

AN INVESTIGATION OF THE LIFE CYCLE OF *MACROZAMIA SPIRALIS* MIQ.

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(Plates xiv-xvii; ninety-three Text-figures.)

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An investigation of the life-history of *Macrozamia spiralis* was begun early in 1938. Two main reasons governed the decision to embark on the work, first, the fact that up to that time little had been published regarding this particular genus, and second, notwithstanding the voluminous literature on cycads in general, many important phases in their life histories remain to be described. In 1939 the authors presented a preliminary account of megasporogenesis in *Macrozamia spiralis*, and since then Baird, A. M. (1939), has published the results of her researches on *Macrozamia Reidlei*. Other investigators, namely, Chamberlain (1913) on *Macrozamia Moorei* and Light (1924) on *Macrozamia Fraseri*, have made contributions to our knowledge of the genus, but it is believed that the present comprehensive account embodies many facts of particular interest, relative not only to *Macrozamia*, but to cycads in general. Only those features which are new, or of special significance, are stressed, although other facts necessary to preserve continuity, and to make comparison with other genera are included.

#### Distribution.

The genus *Macrozamia* is confined to Australia and, in those districts in which it is most abundant, is the dominant plant over very considerable areas. It then forms a much more prominent feature of the flora than either of the other endemic genera, namely, *Bowenia* and *Cycas*.

*Macrozamia spiralis* is widely distributed throughout the eastern coastal areas, extending from southern Queensland almost to the southern limit of New South Wales, a distance of approximately a thousand miles, and in number of individual plants probably exceeds that of any other cycadean species. Typically, it occurs in vast numbers in open eucalyptus forests from just above sea-level to a distance of several hundred yards inland. Occasionally, the species is encountered in isolated areas, each supporting some hundreds of plants, at a distance up to ten or fifteen miles from the coast, but, beyond that, this species is rare, although several small societies have been found as much as thirty miles inland.

#### Habit and Habitat.

*Macrozamia spiralis* finds its maximum expression on sand-dunes adjacent to the sea, or on ridges of light sandy soil formed by the disintegration of the underlying sandstone, or by wind-borne shore sand. In such an environment only the major portions of the leaves and the cones appear above ground. The lower parts of the petioles, the younger leaves, and the organic apex are buried in the soil, as are, of course, the thick tuberous stem and deep root system (Pl. xiv, figs. 2, 3).

More rarely a society flourishes on hard quartzite or sandstone ridges, but then the resistant nature of the subsoil—chiefly rock fragments—retards penetration, and the stem, encased in an armour of leaf-bases, rises above the ground exposing a trunk up to four feet high (Pl. xiv, fig. 4).

Such plants are almost as massive and well-developed as those on softer soils. A comparative study of plants at various stages of development indicates that the continued burying of the apex in the softer soils is due to the action of contractile roots (Text-figs. 89-93). In hard stony situations this contractile force is evidently unable to overcome the resistance of the sub-soil, and so the stems protrude well above ground level (Pl. xiv, figs. 4, 5).

A well-developed plant on the quartzite, then, may have a trunk protruding as much as four feet above the soil level, the extent of the region exposed being in direct proportion to the difficulty of penetration, while the lower part of the stem, and the root system, may extend for two or three feet underground. Accordingly, as is obvious in Plate xiv, figure 5, this form is more attenuated than that characteristic of the softer soils. The protection afforded the exposed stem by the stout persistent leaf-bases is of very decided value as a defence against the heat engendered by bush fires, which are liable to occur frequently on such a dry habitat. Even under the driest conditions the tissues are well supplied with water, and, if a cavity be cut in the sappy tissue just below the stem apex, moisture will gradually accumulate in the hollow.

The plants are dioecious. The number of cones produced during any one season by a staminate plant (Pl. xiv, fig. 3) is on the average in excess of that produced by an ovulate plant, the usual number for the former being three to four, while in the latter, two to three prevail (Pl. xiv, fig. 2). Extreme numbers for the above are seven to ten and five to six respectively. Generally, the dimensions of the cones on plants growing in soft soils are slightly greater than those produced by plants on the stony ridges.

Staminate and ovulate plants possess a similar appearance, and in the field can only be distinguished with certainty by the presence of cones, or by the occurrence of seeds, testas, or seedlings around the trunk of ovulate plants.

#### *Strobili.*

About the beginning of March the cones first become visible amid the crown of leaves. The majority of plants produce cones at the same time and these develop at about the same rate, but individual plants may lag a week or two behind their fellows.

Mature microsporangiate cones, that is, cones at the time of shedding pollen, have an average length of 38 cm. and a diameter of 10.2 cm. The corresponding dimensions of a seed cone are 35.6 × 19.0 cm., but a maximum measurement of 45 × 18 cm. with a weight of 5.77 kg. has been recorded by the writers. Some half dozen mature seed-cones, taken at random, had the following weights: 5.77 kg., 4.31 kg., 4.1 kg., 3.06 kg., 2.608 kg., and 2.154 kg., giving an approximate average weight of 3.6 kg. It is to be noted that in all these data the peduncle has been excluded. The youngest megasporangiate cone, collected on 27th December, 1938, weighed only 0.6 gram, while the microsporangiate cone of the same date weighed 0.7 gram. These records represent the result of field observations ranging from two hundred miles south to thirty miles north of Sydney.

The microsporangiate and megasporangiate cones originate about the same time. The seed cone takes about eighteen months to mature, while the microsporangiate cone sheds its pollen some five months earlier. It is estimated that cones are almost exactly twelve months old at the time of pollination, but a further five to six months elapse before the absciss layer, formed near the base of the megasporophyll, breaks down to permit the collapse of the cone and the dispersal of the seeds.

*Macrozamia spiralis* grows abundantly in the vicinity of Sydney. This fact makes it relatively easy to collect material at regular intervals over a period covering the phases of reproduction. As a general rule, fortnightly intervals were regarded as satisfactory, but during the more critical phases the intervals between successive collections were considerably reduced, until, in tracing megasporogenesis, fertilization and embryogeny, collections were made twice weekly.

The more mature cones were easily harvested, but the younger, such as those showing sporophyll primordia and megasporogenesis, are deeply sunken amid the apical leaves; consequently a more elaborate method of removal had to be adopted. This method is described fully in later pages.

#### *Technique.*

The material was fixed either in strong chromo-acetic solution or in Fleming's fluid, and having been transferred to paraffin wax was microtomed in the usual way.

Three different staining methods were used: (a) Haedenhain's iron-alum haematoxylin method, destaining with picric acid; (b) Newton's iodine method; and (c) Fleming's triple stain.

The first-named method was used throughout, the others only in cases where their adoption conferred some special advantage.

TABLE I.  
*The Megasporangiate Cone.*

With the exception of the first two sets of readings, all data refer to cones from which the peduncle had been removed.

| Date.    | Dimensions (cm.). |          | Weight (grams). | Sporangial Features.  | General Observations.   |
|----------|-------------------|----------|-----------------|---|---|
|          | Length.           | Breadth. |                 |   |   |
| 27/12/38 | 1.5               | 0.8      | 0.6             | Sporangium just visible to naked eye.                                     | Cones hidden amid leaves near stem apex.  |
|          | with peduncle     |          |                 |   |   |
| 20/1/39  | 3.5               | 1.1      | 1.3             |   | do.   |
|          | with peduncle     |          |                 |   |   |
| 31/1/39  | 3.8               | 1.5      |                 | Megaspore mother-cell stage to megaspore formation and young gametophyte. | do.<br>Integument up to shoulder of nucellus.   |
| 11/3/39  | 12.0              | 3.7      |                 | Early free nuclear stage of gametophyte.                                  | Apices of cones just visible amid vegetative leaves; nucellus enclosed by integument.           |
| 14/4/39  | 16.3              | 4.8      |                 | Advanced free nuclear stage of gametophyte.                               | Cones protruding about three inches.  |
| 13/6/39  | 17.2              |          |                 | Wall formation in gametophyte almost completed.                           | Prominent nucellar beak protruding far into micropyle.  |
| 10/7/39  | 19.6              | 7.5      |                 | Wall formation in gametophyte completed.                                  | Gametophyte cells under high pressure and at jelly stage.                                       |
| 16/8/39  | 19.8              | 7.6      |                 |   | Archegonial initials present.   |
| 3/9/39   | 23.0              | 10.2     |                 | Solid gametophyte tissue.   | Archegonia with neck cells beginning to enlarge.  |
| 2/10/39  | 25.4              | 11.3     |                 |   | Some ovules near base and apex of cone have aborted.  |
| 29/10/39 | 25.6              | 14.2     |                 | Gametophyte measures 1.7 cm. $\times$ 1.2 cm.                             | Archegonia arranged in a ring in distinct archegonial chambers. Ovules assume pale pink colour. |
| 12/11/39 | 28.0              | 14.9     |                 | Archegonia enlarged and highly vacuolate.                                 | Sporophylls separated; pollination completed; microspores in shallow pollen chamber.            |
| 10/12/39 | 28.5              | 14.9     |                 | Neck cells protruding into archegonial chamber.                           | Integuments becoming stony. Sporophylls more widely separated.                                  |
| 31/12/39 | 38.1              | 20.3     | 3629            | Archegonia in deep chamber; male gametophyte almost mature.               | Ovules bright scarlet.  |
| 14/1/39  | 41.7              | 19.0     | 5670            | Grand period of fertilization.  | Specially long cone; average wgt. of 6 cones was 3742 gm.                                       |
| 29/1/39  | 30.4              | 17.4     |                 | First division of zygote.   | Cones vary greatly in size; ovule-bearing region of sporophyll now very massive.                |

TABLE I.—*Continued.*  
*The Megasporangiate Cone.*—Continued.

| Date.  | Dimensions (cm.). |          | Weight (grams). | Sporangial Features.   | General Observations.  |
|--------|-------------------|----------|-----------------|--|--|
|        | Length.           | Breadth. |                 |  |  |
| 4/2/40 |                   |          |                 | Proembryos showing incipient wall formation.   | Cone axis elongates with separation of sporophylls; abscission layer apparent.             |
| 5/3/40 |                   |          |                 | Embryos at various stages up to appearance of the two cotyledons.  | Sporophylls detached from cone axis owing to collapse of abscission layers.                |
| 1/6/40 |                   |          |                 | Average number of viable seeds per cone is 126.<br>Average number of aborted ovules per cone is 15.1 per cent. | Number of sterile megasporophylls at apex and base respectively of cone averages about 20. |

TABLE II.  
*The Microsporangiate Cone.*

All data, except first reading, have been made from cones with peduncle removed.

| Date.    | Dimensions (cm.). |          | Weight (grams). | Sporangial Features.   | General Observations.  |
|----------|-------------------|----------|-----------------|--|--|
|          | Length.           | Breadth. |                 |  |  |
| 28/12/38 | 1.9               | 0.8      | 0.7             |  |  |
|          | with peduncle     |          |                 |  |  |
| 3/2/39   | 6.0               | 2.1      | 11.0            |  |  |
| 11/3/39  | 9.0               | 2.6      |                 | Walls and sporogenous cells differentiated. Tapetum not evident.     | Cones hidden amid apical leaves, sori of 3 to 4 sporangia, crowded on lower surface.   |
| 14/4/39  | 12.0              | 3.5      |                 | Sporogenous cells and tapetum present.                               | Cone tips visible amid apical leaves.  |
| 13/6/39  |                   |          |                 | Dehiscence mechanism differentiated; spore mother-cells in prophase. | Cones conspicuous amid apical leaves; sporophylls closely packed; distinct line of dehiscence.   |
| 10/7/39  | 18.5              | 8.4      |                 | do.  |  |
| 6/8/39   | 20.5              | 8.6      |                 | Heterotypic division at metaphase, to tetrad formation.              | Slight separation of sporophylls by elongation of cone axis.   |
| 17/9/39  | 30.0              | 13.5     |                 | Young microspores.   | Sporophyll separation more pronounced.   |
| 12/11/39 | 38.5              | 12.5     |                 | Gametophyte mostly at three-nucleate stage.                          | Sporophylls widely separated; dehiscence of sporangia in progress or completed.  |
| 10/12/39 | 32.9              | 7.0      |                 | Male gametophyte developing in pollen chamber.                       | Empty sporangia; cone drying out and contracted.   |
| 1/6/40   |                   |          |                 | Average number of sporangia per sporophyll is 342.                   | Average number of sporophylls per cone is 345.<br>Number of sterile sporophylls at base and apex of cone respectively averages about 20. |

*The Megasporangiate Cone.*

The youngest cones were procured on 27th December, 1938. Their average dimensions including peduncle were length 1.5 cm. and breadth 0.8 cm., while the weight was 0.6 gram. The ovulate plant typically produces two such strobili, but three or four are not uncommon. Any number beyond this is rare. The cones arise laterally around the growing point in the manner indicated in Plate xvi, figure 11, and are approximately of the same age and dimensions.

The apex of the plant is persistent, and near the organic apex bears numerous whitish-yellow leaves of very soft texture which, like the sporophylls, arise in acropetal succession and are spirally arranged. These young leaves are so delicate that they are unable to support their own weight, being held in position by the enfolding leaves, which, with age, assume a stiffer texture. The cones are approximately at the same level, and each is ensheathed in, and protected by, a large number of spirally arranged, specialized, vegetative leaves, fawn to brown in colour and bearing on the outside a dense soft woolly covering of hairs. The whole forms a bud-like structure about twelve inches in height. The outer protective leaves terminate in long spine-like tips (Text-figs. 3, 4, 5; also Pl. xvi, figs. 11, 12). Each strobilus, then, is most efficiently guarded against desiccation and sudden changes in temperature. Text-figure 2 gives some idea of the shape and size of one of these minute cones, while Text-figure 1 depicts a strobilus, amid its protective leaves, the upper parts of which have been excised, in order to expose the tip of the cone. Even in the youngest cones inspection with a lens of the basal region of the sporophyll reveals two minute protuberances, each the primordium of a megasporangium.

Thereafter, during each successive month, cones were collected and data relative to their gross development recorded. For ease in reference, and to facilitate comparison, the results obtained have been tabulated and presented in condensed form in Table i, where the various developmental stages are set out. Comparison with Table ii permits a ready appreciation of the relative rates and stages of growth of the megasporangiate and microsporangiate cones.

The difficulty in obtaining the more minute cones is well known to all familiar with cycads, and is attested by the paucity of information regarding them. Accordingly, it may not be unprofitable to indicate how, in the case of *Macrozamia spiralis* at least, one may detect their presence with certainty, and so prevent unnecessary destruction of plants, not to mention vexatious waste of time and labour.

In the first place, plants which bear exposed cones are avoided, as experience has shown that in no case have these begun the production of new cones—an interval of a year or more elapses. Seedlings, mature seeds or empty testas indicate the carpellate plant. Next, the mature leaves of an approved specimen are forced downward and outward as far as possible, in order to expose the younger central leaves. These in turn are carefully separated, and the investigator looks for the hard brown tips of the protective leaves encircling each cone. Only the sharp brown withered-looking tips will be apparent. One or more groups of such spines may be observed, and these in a position lateral to the region producing the young, apical, yellowish, vegetative leaves. On account of their close packing, it is not difficult to distinguish such groups from the now widely-spread persistent, protective leaves, associated with defunct cones of some previous season. Thereafter, a trench some two feet deep is dug about half-way around the plant so as to expose the tuberous region and persistent leaf-bases. Then, using a sharp axe, slices roughly tangential and vertical are chopped gradually away, until the central region is approached. In this way the characteristic protective leaves of the young cones are exposed, and by pushing these aside the strobili may be excised without injuring the central growing region (Pl. xiv, fig. 6). The soil may then be replaced, and the plant given an opportunity to recover.

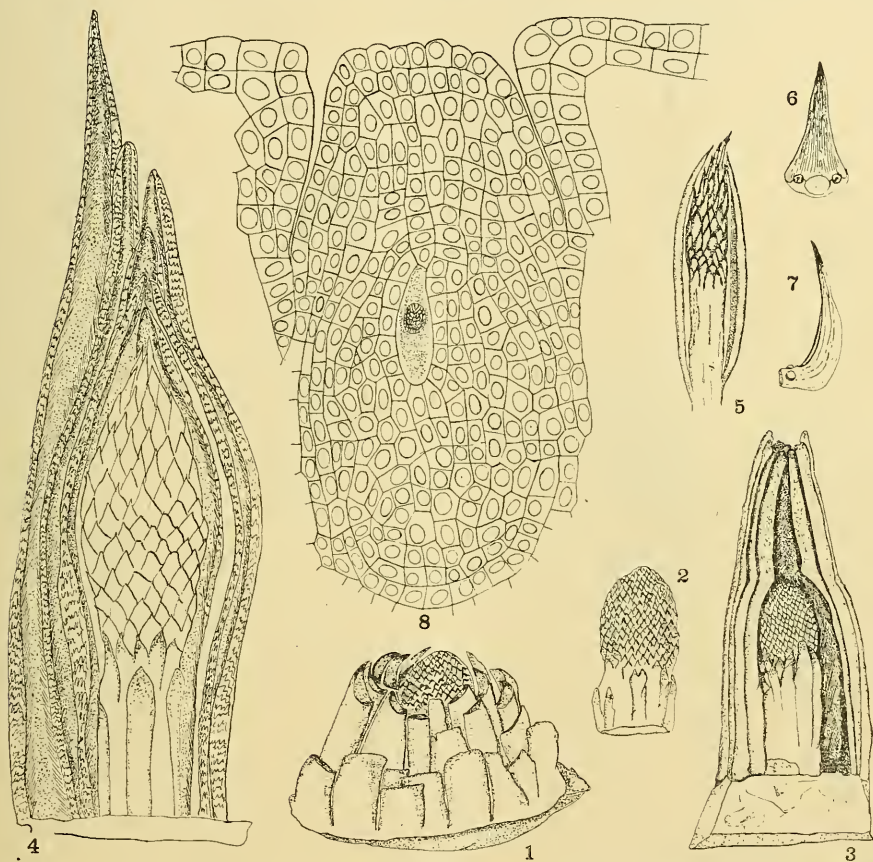
*Megasporogenesis.*

The megasporophylls, as in all cycads, are arranged spirally and in acropetal succession (Text-fig. 4). By careful dissection of the very young cones referred to above,

the individual sporophylls, each bearing two minute megasporangia marginal in origin and adjacent to the cone axis, were separated (Text-figs. 6, 7).

A median vertical section of such a sporangium revealed the presence of a megaspore mother-cell, deeply sunken in the tissue of a massive nucellus which, in turn, was partly enclosed by the young integument (Text-fig. 8).

The mother-cell was most conspicuous on account of its size, prominent nucleus, and typically elongated shape. In its cytoplasm was embedded a large number of starch grains, specially abundant towards the chalazal end (Text-fig. 9). Examination of the megaspore mother-cell, contained in each of a considerable number of sporangia, from the same and from different cones, revealed the nucleus as almost always in the



Text-fig. 1.—Top of minute ovulate cone apparent amid the overlapping protective leaves, the upper portions of which have been excised. 28 Dec., 1939.  $\times 1.6$ .

Text-fig. 2.—Another cone from same plant as in previous figure showing fertile zone and peduncle bearing several scale leaves. 28 Dec., 1939.  $\times 1.6$ .

Text-fig. 3.—A microsporangiate cone showing scale leaves growing from basal region of peduncle, the whole enclosed in a chamber formed by the overlapping and closely packed protective leaves. 28 Dec., 1939.  $\times 1.6$ .

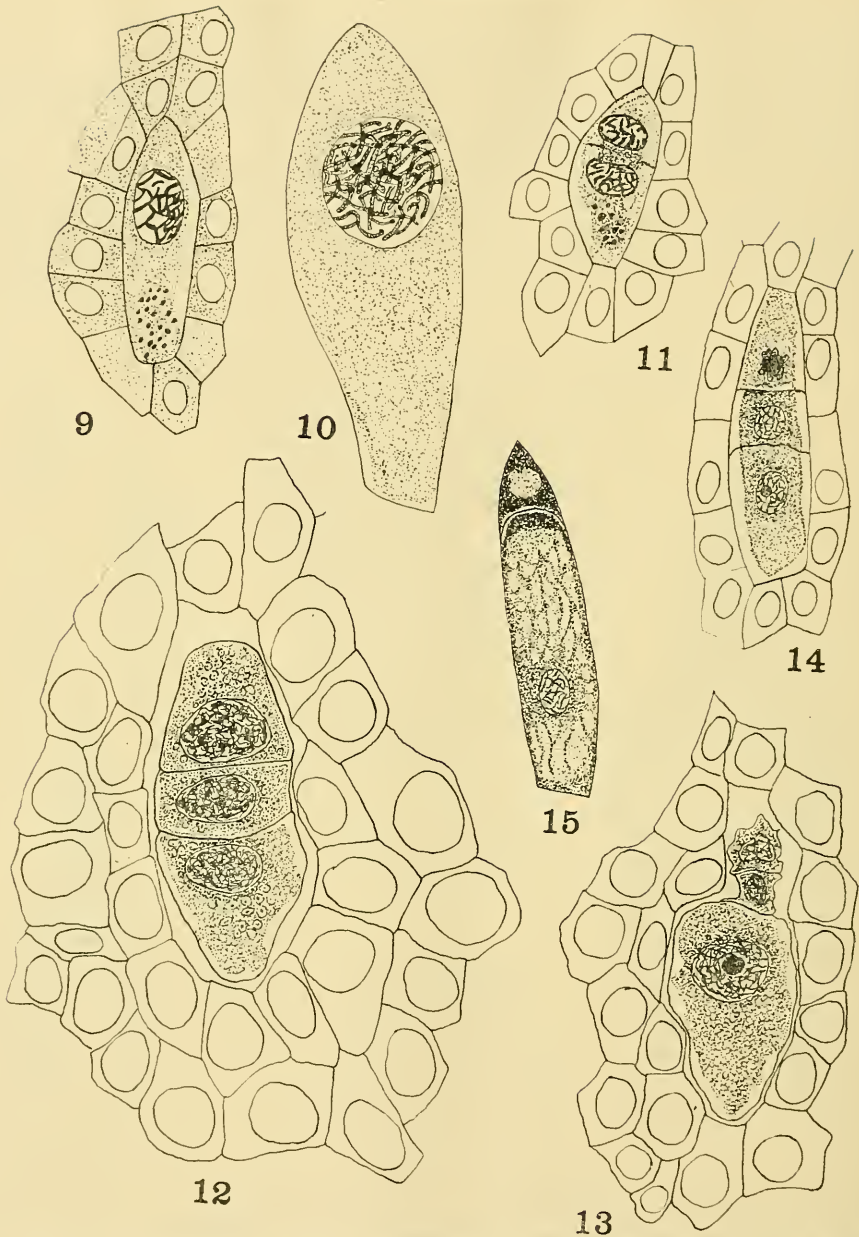
Text-fig. 4.—Lateral megasporangiate cone, bearing scale leaves and enclosed by protective leaves. 20 Jan., 1940.  $\times 1.6$ .

Text-fig. 5.—Ovulate cone five weeks older than that depicted in Text-figure 2. All the protective leaves except two have been removed. 31 Jan., 1940.  $\times 0.4$ .

Text-fig. 6.—Adaxial view of megasporophyll showing marginal position of the two young ovules. 20 Jan., 1940.  $\times 4$ .

Text-fig. 7.—As above but in profile. 20 Jan., 1940.  $\times 4$ .

Text-fig. 8.—Median longitudinal section of very young megasporangium partly enclosed in young integument. The relatively large deeply-sunken cell with conspicuous nucleus is interpreted as the megaspore mother-cell. 31 Jan., 1939.  $\times 34$ .



Text-fig. 9.—Longitudinal section of megaspore mother-cell embedded in nucellar tissue. Starch grains are apparent at end adjacent to chalaza. 31 Jan., 1939.  $\times 490$ .

Text-fig. 10.—Another megaspore mother-cell. The chromatin is segmenting and the nucleus is evidently about to undergo division. 31 Jan., 1939.  $\times 800$ .

Text-fig. 11.—Binucleate condition resulting from division of nucleus at stage represented in Text-figure 9 or 10. A thin wall separates the nuclei, and starch grains are present in lower cell. 31 Jan., 1939.  $\times 490$ .

Text-fig. 12.—A three-nucleate condition attained by division of the nucleus adjoining the chalaza in previous text-figure. 31 Jan., 1939.  $\times 710$ .

Text-fig. 13.—View of the persistent functional megaspore which has increased in size. The sister megaspore, and the third or micropylar nucleus are disintegrating, but the original dividing walls are still evident. 31 Jan., 1939.  $\times 710$ .

Text-fig. 14.—Another view of the three-celled stage in which the nucleus adjacent to the micropyle is beginning to disintegrate.  $\times 710$ .

Text-fig. 15.—The functional megaspore capped by the remains of the two evanescent cells. 31 Jan., 1939.  $\times 490$ .

resting condition. However, in a few cases (see Text-fig. 10), the chromatin had thickened and commenced segmentation, while in one particular case, individual chromosomes were recognized, although their exact number was not determinable. Unfortunately, despite persistent search, the material sectioned did not reveal the spindle formation which should normally ensue. None the less, the subsequent binucleate stage was identified (Text-fig. 11), the nuclei being separated by a thin wall. No wall, however, was recorded, for the corresponding stage, by Smith (1910) in the case of *Zamia floridana*. Thereafter, the chalazal nucleus divided, and a row of three cells resulted (Text-fig. 12), as compared with the four for *Zamia* (Smith, 1910) and three for *Stangeria paradoxa* (Lang, 1900). Numerous preparations were made and examined, but in no case was the micropylar nucleus found to divide. Accordingly, the writers are of opinion that the three walled-cells in a row represent the full progeny of the spore mother-cell.

Subsequently, the chalazal cell enlarged, and the nuclei of the other two cells gradually disintegrated, the evanescent cells forming a densely-staining cytoplasmic cap at the micropylar end of the functional and enlarging megaspore (Text-figs. 13, 14, 15). Clearly, the surviving nucleus was that of the functional megaspore.

At this stage, it is appropriate to call attention to the fact that Professor J. C. Chamberlain, referring in the course of correspondence to the writers' earlier account of megasporogenesis (Brough and Taylor, 1939), made the following pertinent observation: "You speak of a row of three megaspores; when you stop to think about it, only two of the three are megaspores; the third, unless it divides, is still a 2x structure and has not yet reached the megaspore stage."

Accordingly, in the present account, the writers have refrained from referring to the three cells in question as megaspores, although this procedure does not necessarily imply abandonment of the view previously expressed.

Only a demonstration of the actual chromosome sets involved will determine with certainty the correct constitution of the nuclei concerned. This, in turn, will furnish an answer to the critical question as to when reduction division actually takes place, and so finally decide which cell is the spore mother-cell and which cells are megaspores.

#### *Female Gametophyte.*

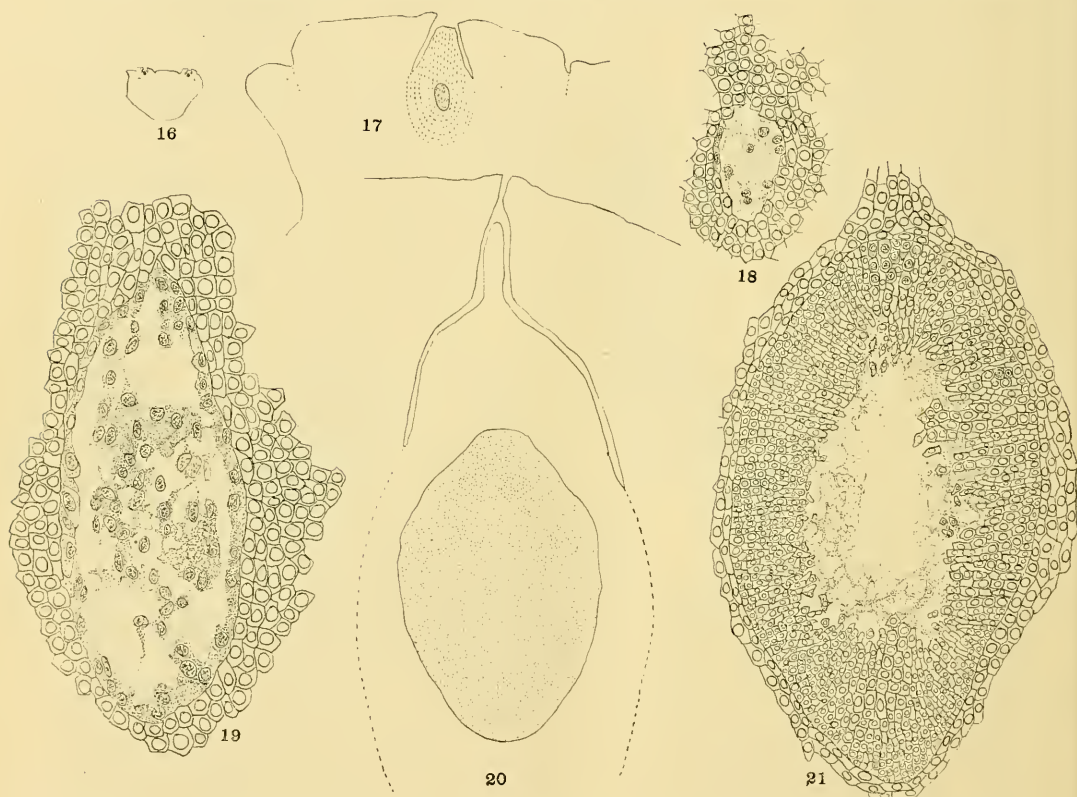
The functional megaspore without any resting period develops into the young gametophyte (Table i). Text-figure 16 shows a megasporophyll in surface section bearing two ovules, each of which contains such a gametophyte with several nuclei embedded in the cytoplasm within the megaspore membrane. A later stage under higher magnification is depicted in Text-figures 17 and 18. In these cases the cytoplasm is slightly vacuolate, and the number of nuclei has increased. Text-figure 19 illustrates portion of an even more advanced stage. The gametophyte has increased greatly in size, while the numerous nuclei are embedded in increasingly vacuolated cytoplasm. The section is not median, hence the prothallus is shown partly in tangential view. It is evident, however, that wall formation has commenced in contact with some regions of the megaspore membrane. Further development proceeds rapidly (vide Table i) until the gametophyte is represented by a layer of tissue lining the megaspore membrane, and enclosing a large central vacuole. Thereafter, wall-formation is continued and centripetal growth proceeds apace (Text-figs. 20 and 21) until finally a continuous mass of cells, rich in starch, fills the entire space within the membrane.

At this stage the jelly-like tissue is under exceedingly high pressure and, when punctured, the contents spurt out with considerable force.

#### *The Archegonium.*

The course of development of the archegonium was found to correspond in the main with accounts already published of this structure in other cycads. The archegonial initials were discernible about the time that wall-formation in the gametophyte was completed. In the apical region of the prothallus an initial is recognized by its larger dimensions relative to the surrounding cells, and prominent nucleus (Text-fig. 22). This initial enlarges, the nucleus dividing to give an outer or primary neck-cell and an inner cell (Text-fig. 23). The former then divides by an anticlinal wall, thus

initiating the two young neck-cells (Text-figs. 24 and 25). The inner cell, meanwhile, increases enormously in size, the cytoplasm becoming denser but containing very numerous vacuoles of varying dimensions, which impart the frothy appearance depicted in Text-figure 26. During this period the contiguous cells, distinguished by the regularity of their arrangement and the density of their contents, become conspicuous and constitute the archegonial jacket (Text-figs. 23-26). While these changes are being effected, a depression, known as the archegonial chamber, is forming at the micropylar end of the gametophyte. Archegonia are visible on the floor of this chamber (Text-fig. 27), where the neck-cells of five archegonia are clearly in evidence. In this connection, it may be mentioned that the writers encountered several cases of malformed or irregular chambers. Text-figures 28 and 29 illustrate two typical cases.



Text-fig. 16.—Section cut parallel to adaxial surface of sporophyll to show the marginal position and general structure of the two young ovules. The integument, nucellus and position of the young gametophyte are indicated. 31 Jan., 1939.  $\times 2\frac{1}{2}$ .

Text-fig. 17.—One of the ovules in previous figure under a higher magnification. 31 Jan., 1939.  $\times 40$ .

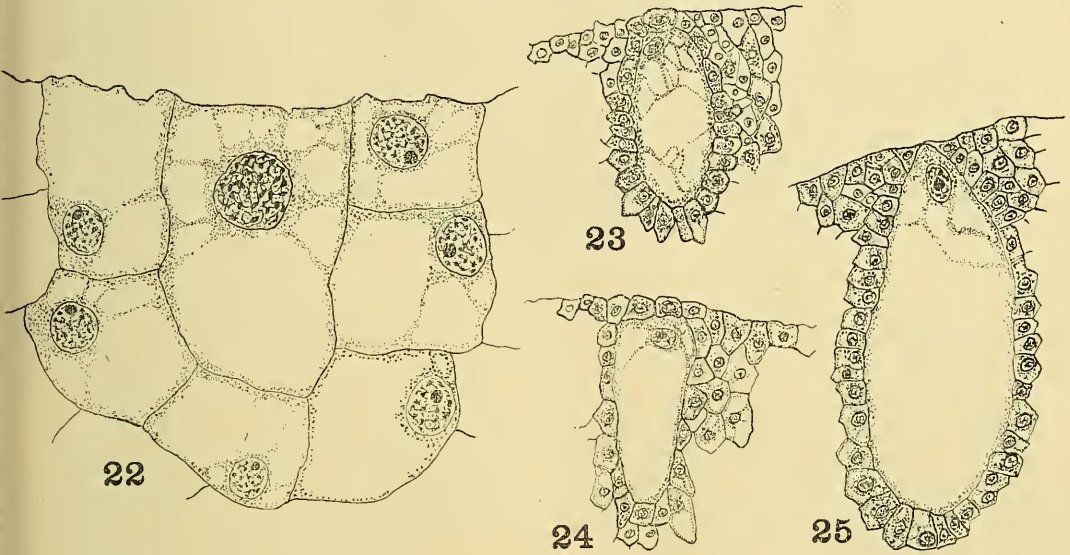
Text-fig. 18.—High-power study of part of ovule depicted in Text-fig. 16, showing young female gametophyte at early free-nucleate stage. The nuclei are embedded in a peripheral layer of cytoplasm enclosing a central vacuole. 31 Jan., 1939.  $\times 180$ .

Text-fig. 19.—A somewhat later stage of development of the prothallus cut longitudinally. The gametophyte has increased greatly in size while the numerous nuclei are embedded in the vacuolated cytoplasm. Wall-formation has commenced against some regions of the megaspore membrane. Section is more or less tangential. 11 Feb., 1939.  $\times 180$ .

Text-fig. 20.—Median longitudinal section of ovule showing integument, nucellus with long beak protruding into micropyle, and female gametophyte. Note great increase in size since 31 Jan., 1939.  $\times 40$ .

Text-fig. 21.—Median longitudinal section through female gametophyte of previous text-figure. The regular centripetal growth of the original peripheral cells is strongly emphasized, and the invasion of the central vacuolate region is about two parts completed. The tissue within the original megaspore membrane has increased enormously in size and is of a jelly-like consistency.  $\times 180$ .

In the first, the chamber bearing eight archegonia is crescent-shaped with a raised part in the centre, while in the other the archegonia are in two separate compartments, one partial chamber with four and the other with three. Such chambers are not regarded as having any special significance.



Text-fig. 22.—Median longitudinal section of archegonial initial, which is vacuolate, has a large nucleus, and is of greater dimensions than the contiguous cells which later form a conspicuous jacket. 16 Aug., 1939.  $\times 710$ .

Text-fig. 23.—Median longitudinal section of a young archegonium showing primary neck-cell just before division and also the central cell; the nucleus of the latter adjoins the primary neck cell. The cytoplasm of the central cell shows numerous vacuoles. An archegonial jacket is evident. 16 Aug., 1939.  $\times 100$ .

Text-fig. 24.—Median longitudinal section of a slightly older archegonium. In this case, the primary neck-cell has divided, giving rise to the two neck-cells of the mature archegonium. 16 Aug., 1939.  $\times 100$ .

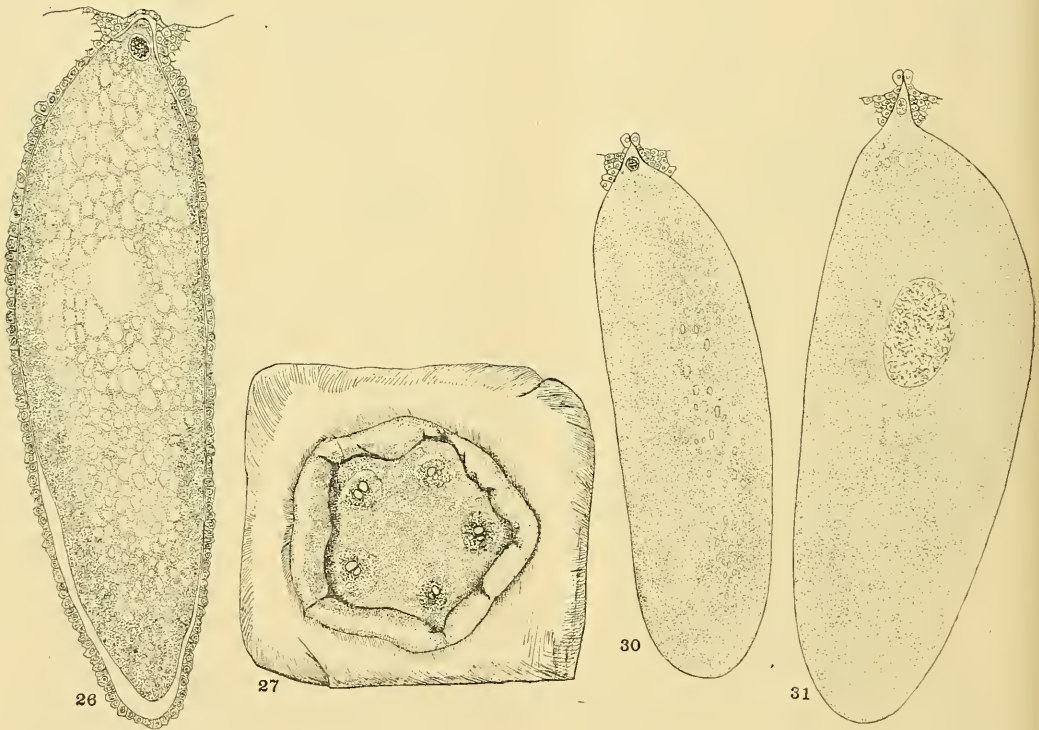
Text-fig. 25.—An archegonium, slightly older than that depicted in previous figure. The section is not quite median and the neck-cells are not in evidence. The archegonial jacket is now clearly differentiated. 17 Sept., 1939.  $\times 100$ .

At the stage when the inner cell of the archegonium shows maximum vacuolation, the nucleus is characteristically located close to the two neck-cells which, up to this time, are relatively insignificant in size. However, the immediately subsequent development is marked by enormous increase in dimensions of the neck-cells which now protrude bodily into the chamber. Meanwhile the vacuoles of the inner cell have been steadily decreasing in number and size (Text-fig. 30).

The nucleus of the large inner cell does not long retain its identity for, on the same cone, cases are found where this condition, owing to mitotic division of the single nucleus, has been succeeded by the two-nucleate phase. These two nuclei are named the ventral canal-nucleus and the egg nucleus respectively. The former remains small and occupies the position originally held by the parent nucleus, while the latter has not only increased enormously in size, but has taken up a position about the centre of the cell. In addition the cytoplasm has become much more evenly granular, while the fast-disappearing vacuoles are relatively few in number (Text-fig. 31).

The dilated neck-cells, in longitudinal and transverse section, are shown in Text-figures 32 and 33 respectively. Each is vacuolate and provided with a prominent centrally placed nucleus. The relative thinness of the uniform wall is emphasized by comparison with the conspicuous egg-membrane, which has been undergoing a steady process of thickening during development. This thickening is interrupted by numerous

wide pits, through which coarse connecting strands of cytoplasm extend. Typically, the primary neck-cell divides once, but the process need not stop there, as is revealed by the fact that this investigation brought to light certain cases in which the subsequent division of the two neck-cells resulted in the formation of a neck consisting of four cells. Text-figure 34a shows such a neck in transverse section, while Text-figure 34b illustrates the same structure but with the cells separated in such a manner as to enclose a central canal. Necks comprising more than two cells have already been recorded for *Encephalartos villosus* by Sedgwick (1924), who figures as many as six. In one case the writers found a single chamber containing four archegonia, each with a neck composed of four cells. This may be of some significance in view of the general similarity between *Macrozamia* and *Encephalartos*, a similarity which may find its origin in a close relationship. A computation of the number of archegonia occurring within different chambers in numerous ovules selected from various cones gave the following results:



Text-fig. 26.—Later stage in development of the archegonium. Two neck cells are evident and their nuclei are relatively small in comparison with the large nucleus of the central cell which lies near the neck. The very numerous vacuoles give the cytoplasm a frothy appearance. A very pronounced layer of nourishing cells, forming the jacket, invests the central cell. 29 Oct., 1939.  $\times 50$ .

Text-fig. 27.—Surface view of the archegonial chamber at apex of female gametophyte. The rim of the chamber slightly overhangs the cavity, on the floor of which five protruding twin neck-cells are apparent. 8 June, 1939.  $\times 8\frac{1}{2}$ .

Text-fig. 30.—An advanced stage of archegonial development in which the archegonium has almost attained mature size. The vacuoles are considerably reduced in number and size. The neck-cells are dilated and project prominently above the floor of the archegonial chamber, while the nucleus lies in the attenuated apex of the central cell. 21 Nov., 1939.  $\times 28$ .

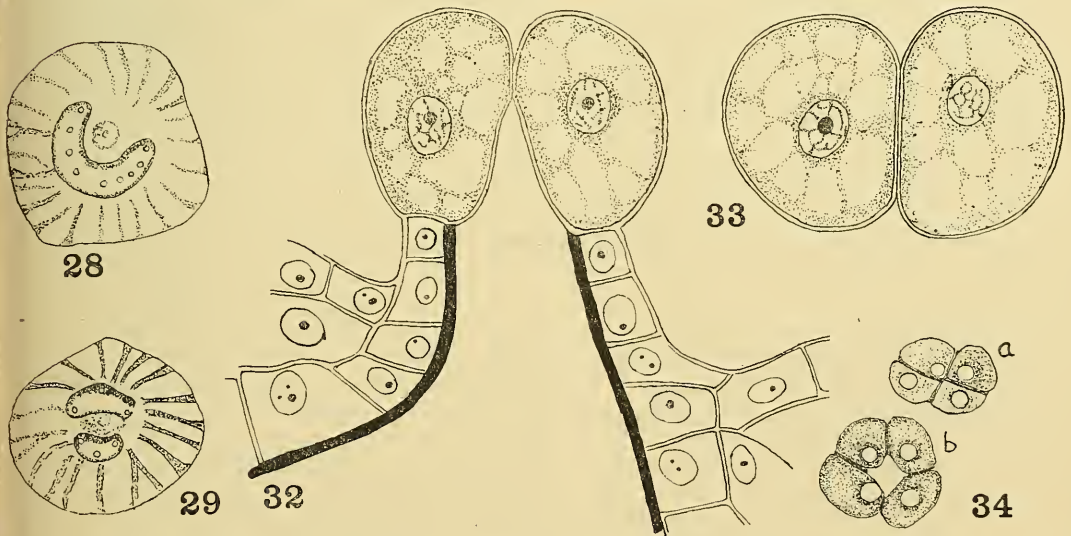
Text-fig. 31.—Median longitudinal section of an archegonium which differs from that of the previous figure in that the central cell nucleus has already undergone division giving the ventral canal nucleus and the enormous egg-nucleus. 21 Nov., 1939.  $\times 28$ .

|   |   |   |   |   |          |
|---|---|---|---|---|----------|
| Percentage of ovules with 3 archegonia = 13.0 |   |   |   |   |          |
| "   | " | " | " | 4 | " = 39.5 |
| "   | " | " | " | 5 | " = 40.0 |
| "   | " | " | " | 6 | " = 6.0  |
| "   | " | " | " | 7 | " = 1.0  |
| "   | " | " | " | 8 | " = 0.5  |
| <hr/>   |   |   |   |   |          |
| 100.0   |   |   |   |   |          |
| <hr/>   |   |   |   |   |          |

### *The Microsporangiate Cone.*

The microsporophylls arise in acropetal succession and are arranged spirally on the axis (Pl. xv, fig. 8*b*). They vary considerably in shape and size according to their position on the cone, the most noticeable difference being the greater length of the spine-like terminal portions of the upper sporophylls (Pl. xv, figs. 10*a*, 10*b*). Again, despite the fact that the microsporangiate cone is much less bulky than the megasporangiate (Tables i and ii; and Pl. xv, figs. 8*a*, 8*b*), it none the less bears a much greater number of sporophylls. Actual counts from representative cones of each kind showed the microsporangiate cone to bear from 300 to 450 sporophylls, while the ovulate cone produced only from 70 to 105.

The youngest cones observed were collected on 28th December, 1938. These minute strobili with peduncles attached (Text-fig. 3) had the following average dimensions: length 1.9 cm., breadth 0.8 cm., while the average weight was 0.7 gm. In size and general appearance they somewhat resemble the microsporangiate cones of *Pinus*, and have, near the base of the peduncle, similar scale leaves to protect the very young sporophylls. Typically, the cones occur in groups of three or four, and always in a



Text-fig. 28.—An abnormal crescent-shaped archegonial chamber in surface view. Nine archegonia are indicated. 8 June, 1939.  $\times 1\frac{1}{2}$ .

Text-fig. 29.—Another abnormal archegonial chamber which consists of two subsidiary compartments, each bearing archegonia. 8 June, 1939.  $\times 1\frac{1}{2}$ .

Text-fig. 32.—Median vertical section through two greatly distended neck-cells, whose uniformly thin walls, highly vacuolate nature, and large size are evident. When these cells are fully extended they only impinge over a relatively small part of the opposing areas. 21 Nov., 1938.  $\times 320$ .

Text-fig. 33.—Transverse section through the two neck-cells. 21 Nov., 1938.  $\times 320$ .

Text-fig. 34.—Transverse section of an abnormal neck consisting of four cells investing a short canal, through which, in this case, the sperms probably gain entrance to the egg. (a) shows neck-cells in closed position; (b) shows neck-cells, 10 minutes after opening, with central canal. 10 Jan., 1939.  $\times 70$ .

position lateral to the growing point. Their rate of growth is relatively fast, as exemplified by the entry in Table ii of data relating to a cone collected in early February, by which date the dimensions were, length 6.0 cm., breadth 2.1 cm., while the weight was 11.0 gm.

The above, and all succeeding data, concerning the strobili exclude the peduncle. Again, cones collected in mid-March average 9.0 cm. in length and 2.6 cm. in diameter (Pl. xvi, fig. 13).

At this stage the soral arrangement is evident, there being typically three to four sporangia per sorus, the former number, however, predominating. Inside each sporangium an undifferentiated mass of sporogenous cells is found, but without any evident tapetum.

An appreciation of the subsequent stages in development, terminating in the mature cone, may be gained by a study of the relevant columns in Table ii.

#### *The Microsporophyll.*

A series of photographs illustrating the size of the microsporophyll at different times of the year as compared with the size of the megasporophyll at the same dates is presented in Plate xvi, fig. 15, *a-d*, and Plate xv, fig. 9, *e-h*.

The relatively smaller size of the oldest microsporophyll is due to shrinkage consequent upon drying out after dehiscence of the sporangia and pollen dispersal. Again, the microsporophylls may remain attached to the cone axis for a year or more after pollen distribution, but the megasporophylls normally fall from the axis some five months after pollination, a discrepancy due to the early disintegration of the cells of the prominent abscission layer of the megasporophyll.

#### *The Microsporangium.*

On younger sporophylls, as already indicated, the sporangia occur in soral groups of three or four. On further development, however, such definite arrangement becomes progressively less distinct and finally unrecognizable. This may be explained by displacement and changed orientation of the individual sporangia consequent upon their greater increase in size not being fully balanced by synchronous growth of the sporophyll.

The number of sporangia per sporophyll varies with the position of the sporophyll on the cone. Some idea of the range of this inconstancy was obtained from actual counts which gave the following results:

|  |      |     |
|--|------|-----|
| Average number of sporangia on sporophyll near tip of cone | .... | 275 |
| " " " " " " " middle "                                     | .... | 385 |
| " " " " " " " base "                                       | .... | 255 |

In addition it was observed that the highest number of sporangia encountered on any one sporophyll was 412, while the lowest count was 195.

The shape of the sporangium in surface view varies from round to elliptical, the latter form becoming more pronounced as the pressure due to enlargement of contiguous sporangia increases (Pl. xv, figs. 10*a* and 10*b*).

The line of dehiscence first becomes discernible in early June, and is defined by the differentiation of a band of tissue, four cells broad, which becomes distinct owing to the relatively small size and thinner walls of its component cells (Text-fig. 37).

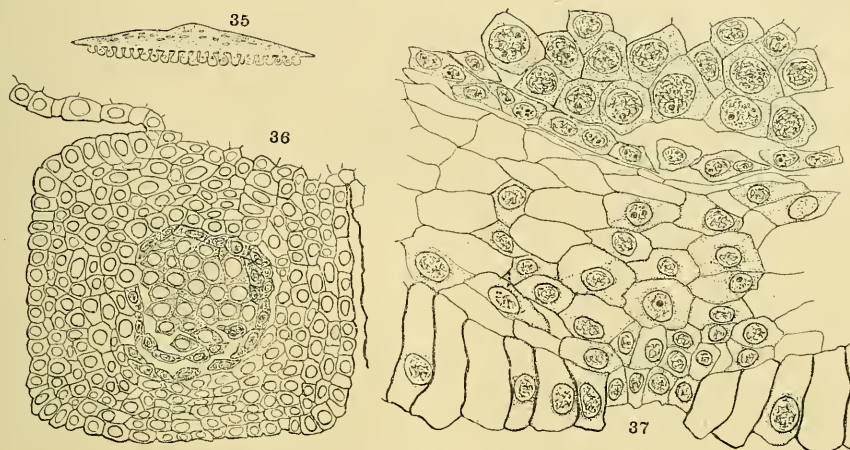
At maturity, usually during late October and throughout November, these cells break down, thus enabling the sporangium to gape widely and so liberate the pollen grains which are shed as a fine, powdery, cream-coloured mass (Pl. xv, fig. 10*b*). This dispersal is continuous over a period of several weeks because the sporangia open progressively throughout October and November, when the pollen gradually sifts out between the sporophylls.

The lightness of the abundant microspores renders them easy of distribution by wind action, which transfers them to the open micropyles of ovules now ready for reception.

In view of the fact that numerous accounts of the development of the cycadean microsporangium—from the stage of the sporogenous tissue to the production of mature

spores—have already been made, for example by Baird (1939), Chamberlain (1909), Lang (1897), and Smith (1907), it will suffice in the present instance to present only the more significant features.

Text-figure 35 is a transverse section of a microsporophyll collected in March. It is cut at right angles to the long axis, and portrays the shape and arrangement of the sporangia in vertical section, as well as their size relative to the thickness of the sporophyll. Numerous mucilage sacs are also in evidence.



Text-fig. 35.—Transverse section of young microsporophyll: hand-cut section showing arrangement of microsporangia on abaxial surface of thick, woody sporophyll. 13 June, 1939.  $\times 2$ .

Text-fig. 36.—Longitudinal section of a young microsporangium showing massive wall of regularly arranged cells in which the line of dehiscence has not yet been differentiated; the central zone of sporogenous tissue is distinct, its cells having large nuclei and dense contents; a zone of tapetal cells already surrounds the central tissue: these cells are tangentially elongated and somewhat irregularly arranged. 14 April, 1939.  $\times 180$ .

Text-fig. 37.—High power study of portion of longitudinal section of an older sporangium. The epidermal cells have differentiated, the majority being large, elongated, and radially thickened. A row of four cells in the central region of the wall, however, has remained small and thin-walled and defines the region of dehiscence. The inner cells of the wall are crushed, the tapetal zone is conspicuous, and encloses the large polygonal thin-walled densely cytoplasmic microspore mother-cells. 13 June, 1939.  $\times 360$ .

The succeeding Text-figure shows a high-power study of a single one of these sporangia cut in a plane at right angles to the sporophyll. The wall, including the tapetum, is seven cells thick. The tapetal cells are elongated tangentially, and usually multinucleate. Although the layer is quite clearly differentiated, it at no stage assumes the prominence of that characteristic of similar structures in angiosperms. The specialized cells, which later determine the line of dehiscence, have not yet been differentiated. Some three months later the spore mother-cells are formed. These at first are close packed (Text-fig. 37) but later float separately in a nutritive matrix.

Their subsequent cytological history up to the formation of the mature microspores was traced, but the sequence of events conformed so closely with those already recorded for the corresponding phases in other cycads that it has not been deemed advisable to recall our observations here.

About the period of meiosis the tapetum commences to break down, as do certain of the inner cells of the wall, but even at maturity the wall is some four to five cells thick.

The abaxial epidermal cells, excluding those which constitute the region of dehiscence, are of a brownish colour with the radial and inner tangential walls considerably thickened, while the epidermal cells of the adaxial surface, that is, the region towards the stalk of the sporangium, are yellowish and thinner walled.

Dehiscence is due to the drying and contraction of these latter cells which exert a downward pull on the indurated cells towards the apex, and this in turn leads to the tearing apart of the band of cells which defines the actual line of dehiscence.

This account agrees with that given for *Stangeria paradoxa* (Lang, 1900) except for the fact that in the case of *Macrozamia spiralis* the band of cells governing the line of dehiscence is four cells broad instead of two as in *Stangeria paradoxa*.

#### *The Early Male Gametophyte.*

As a result of meiosis uninucleate microspores (Text-fig. 39) are normally produced in the microsporangium during late August. On division, which typically occurs during October, this nucleus produces a centrally placed tube nucleus and a second one close to the wall of the spore (Text-figs. 40 and 41). This latter nucleus, usually in November, divides in turn, giving a prothallial cell and a generative cell, each invested by a thin membrane (Text-fig. 42). This stage represents the microspore at the period of dehiscence and dispersal, and is the phase characteristic of the numerous microspores present in the young pollen-chamber (Text-fig. 38). This cavity has been formed by the breaking down of cells in the beaked part of the nucellus which protrudes into the micropyle (Text-fig. 20).

#### *Pollination.*

During November the wind-borne microspores drift down between the megasporophylls which, at this period, have become slightly separated by elongation of the cone axis. At such time, too, the significant observation was made that a tiny drop of fluid appears at the end of each micropyle, as has been explained by Webber (1901) and Chamberlain (1935). The microspores adhere to this liquid which, by its subsequent evaporation, draws the pollen grains down the micropyle to be lodged in the young pollen chamber (Text-fig. 38). The cells, which have broken down to form this chamber, may furnish the liquid referred to. In a single longitudinal section of a pollen chamber (Text-fig. 38) eleven microspores are in view, so that the total number in the complete chamber must be very considerably more.

Baird (1939), in the case of *Macrozamia Reidlei*, is convinced that insects play "an important part in transferring pollen from the cracks between the megasporophylls to the micropyle". During the collection of material of *Macrozamia spiralis* the writers made frequent observations regarding this point, and reached the conclusion that although insects, chiefly beetles, may be encountered amongst the younger vegetative leaves, and even among the sporophylls of both kinds of cones, yet their presence is fortuitous and sporadic.

Furthermore, while pollen which has already been carried by the wind to the ovulate cones may on occasion be transferred by insects from a megasporophyll to the micropyle of an ovule, yet such a rare happening is not to be regarded as of any real significance in the mechanism of pollination which, for all practical purposes, is anemophilous.

Text-fig. 39.—Optical section of a microspore removed from a microsporangium prior to dehiscence. The exine and intine and large central nucleus are indicated. 12 Nov., 1938.  $\times 960$ .

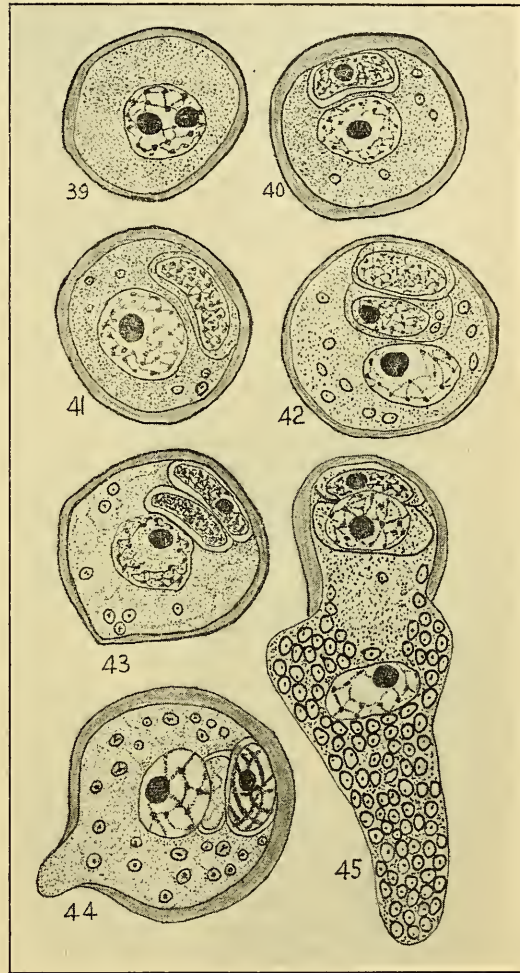
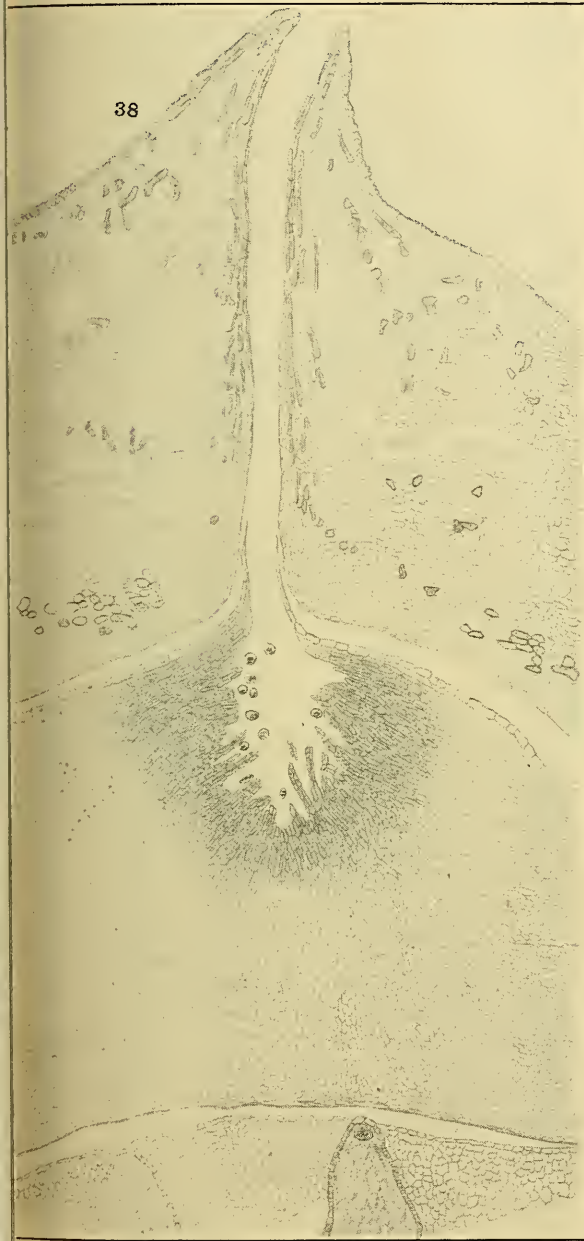
Text-figs. 40, 41.—Sections from material slightly older than that of preceding figure. The single nucleus has divided to produce two nuclei. One, surrounded by cytoplasm and a thin wall, lies against the wall of the spore and represents the prothallial cell, while the other, a spherical nucleus, occupies a central position. Some starch grains are present in the cytoplasm. 12 Nov., 1938.  $\times 960$ .

Text-fig. 42.—Transverse section of microspore at shedding stage. The central nucleus has divided, giving rise to a central generative nucleus and tube-nucleus. Starch grains have become more numerous. 12 Nov., 1938.  $\times 960$ .

Text-fig. 43.—Section of pollen grain within pollen chamber, just after pollination of ovule. The slight swelling on one side indicates the commencement of growth of the pollen tube. 12 Nov., 1938.  $\times 960$ .

Text-fig. 44.—Section of microspore and developing male gametophyte: the intine has ruptured the thinner region of the exine and protrudes as a blunt tube; the starch content has steadily increased. 12 Nov., 1938.  $\times 960$ .

Text-fig. 45.—A later stage of the male gametophyte showing further development of the pollen tube, especially in breadth; the tube-nucleus has moved into the tube which is densely packed with starch grains.



Text-fig. 38.—Median longitudinal section of the upper portion of an ovule during pollination stage. The thick integument contains numerous mucilage ducts, and tannin cells, and is prolonged into a low beak enclosing a long narrow micropyle, which is funnel-shaped at the apex, and lined internally by a definite layer of relatively small secretory epidermal cells. The long, narrow, hardened nucellar beak projects a considerable distance into the micropyle. The cells of the central region of this beak have disintegrated to form the apical narrow passage which lower down expands into the enlarging pollen chamber in which numerous pollen grains at the 3-nucleate stage of the gametophyte are evident.

Below this again is a deep zone of nucellar tissue which caps the female gametophyte, in which are seen portions of two archegonia with young neck-cells and a large central cell with highly vacuolate contents. 12 Nov., 1938.  $\times 40$ .

*The Late Male Gametophyte.*

Further development of the male gametophyte may proceed without any interval of rest, but at any time various stages can be seen in any pollen chamber inspected. Growth is marked by the greater number of starch grains, a small increase in the size of the spores, and protrusion of the intine (Text-figs. 43 and 44) to initiate the pollen tube. A slightly older stage is illustrated in Text-figure 45, in which it is evident that the tube-nucleus has entered the pollen tube, the tip of which has invaded the nucellar tissue approximately at right angles to the long axis of the pollen chamber. As the tube elongates, the position taken by the tube-nucleus is not fixed, but may be anywhere from almost touching the body cell (Text-figs. 46 and 47) to close proximity to the end penetrating the tissue. The latter is the usual location in the final stages of development. As growth proceeds the haustorial portions of the pollen tubes may branch a few times (Text-figs. 53 and 56) so that the nucellar cap becomes completely riddled, the component cells deprived of their contents, and eventually represented by a flattened mass of collapsed cell walls. By this means the pollen tube is well nourished, and that the process is efficient appears from the dense aggregation of starch grains particularly in that region of the gametophyte adjoining the pollen chamber, where marked activity is evident. Division of the nucleus in this region has resulted in the formation of the prothallial cell and the generative cell, which latter, in turn, divides to give the stalk-cell and the body-cell. During this phase the aggregation of starch—which is particularly well defined by Newton's method of staining—is so marked that considerable difficulty may be experienced in following the successive changes, and the limits of the cells concerned. This difficulty is increased by the frequent overlapping of the cells under observation, namely, the prothallial cell, stalk-cell and the body-cell. However, as is indicated in Text-figures 48 (*a*, *b* and *c*), it was established that the prothallial cell and the stalk-cell occupy the tip of the tube which is in part still encased in the exine. The stalk-cell may extend upwards beyond the lower limit of the body-cell. In so doing it, when fully developed, presses into the latter without, however, rupturing the impinging walls concerned (Text-figs. 47*a*, 48*a* and 49). At maturity the prothallial cell has a distinct nucleus, is somewhat elongated, and in shape varies from elliptical to pyriform with the narrower end towards the body-cell (Text-fig. 47*b*). The latter rapidly increases in size and bears a strikingly large nucleus with dense contents. Very soon two blepharoplasts, disposed at opposite poles, become apparent. Each contains several vacuoles which impart a granular appearance to the organ. With each blepharoplast radiating fibrils of cytoplasm become early associated (Text-figs. 47*a* and 49) and eventually the characteristic appearance of the mature body-cell is attained. The orientation of the blepharoplasts is usually in the long axis of the tube, but may be inclined, or even at right angles, as is seen in Text-figure 47*a*, where it has been forced to assume a flattened form owing to the restrictions imposed by the configuration of this particular pollen tube. Meanwhile, the proximal regions of the tubes, densely packed with starch grains, have elongated, increased in diameter, and now hang vertically downward with their tips protruding into the archegonial chamber. A score, or even more, of these tubes may be seen in a single chamber, but the average number is about a dozen. Text-figures 54, 55, 56 and 57 show the nucellar cap with numerous pendent pollen tubes. Ultimately the body-cell becomes orientated so that a line joining the blepharoplasts lies at right angles to the long axis of the pollen tube (Text-fig. 49). The nucleus of the body-cell is very large and rich in chromatin (Text-fig. 48, *a* and *b*), while the blepharoplasts, each with its conspicuous radiating fibrils of cytoplasm, are especially prominent structural features of the cell. Division of the body-cell, which occurs early in January, gives rise to two mother cells, each containing a sperm with spirally arranged ciliated band so characteristic of cycads (Text-fig. 58). The processes leading to the production of such sperms show no unusual features, and have been so often and so fully described that little purpose would be served in recapitulating the successive phases. Pollen tubes dissected from living



Text-fig. 46.—Median vertical section through nucellus showing, on right, the haustorial nature of the vacuolate pollen tubes. The centrally-placed gametophyte shows the stalk cell, body cell and tube-nucleus. 31 Dec., 1938.  $\times 40$ .

Text-fig. 47.—(a) Median longitudinal section of the generative region of an immature male gametophyte. The remains of the exine are still evident, and adjoining is the stalk cell, which conceals the smaller prothallial cell except for the nucleus. Next is the flattened body-cell with its large nucleus and two conspicuous polar blepharoplasts, while beyond is the tube-nucleus. The small supplementary diagram (b) shows outline of the prothallial cell, seen in a different plane. 31 Dec., 1938.  $\times 140$ .

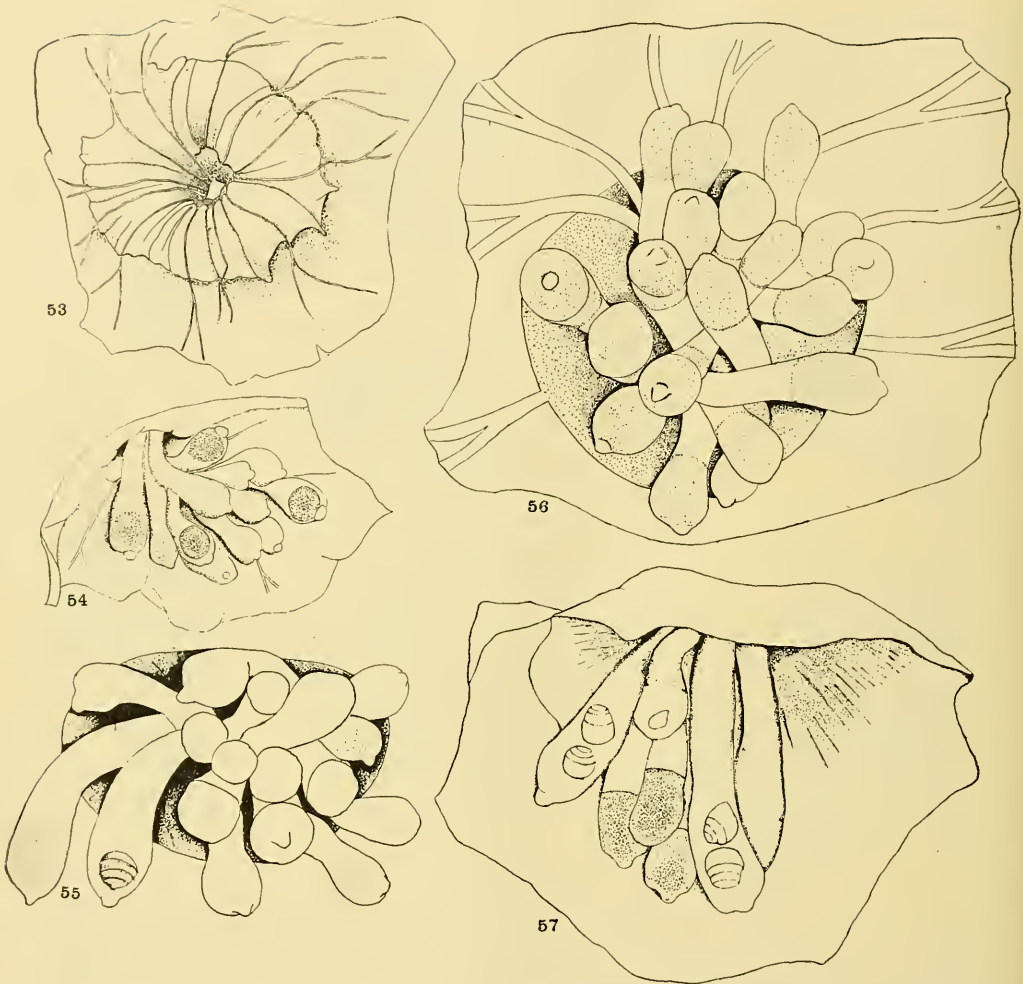
Text-fig. 48.—The generative tips of three male gametophytes in longitudinal section. In (a) may be seen the prothallial cell, the upper part of which overlaps the lower region of the body cell where the nucleus and one of the blepharoplasts are visible. The tip of the pollen tube is crammed with starch grains which tend to cloak the contents; (b) is not median but illustrates the abundant starch and portion of exine, while (c) shows the prothallial and body cells. 12 Nov., 1938.  $\times 140$ .

Text-fig. 49.—This figure shows again the generative tip of gametophyte with the prothallial cell projecting beyond and behind the body cell, the latter possessing two blepharoplasts with radiating fibres and a prominent nucleus with a large nucleolus. 31 Dec., 1938.  $\times 140$ .

Text-fig. 50.—This represents the tips of two male gametophytes pendent from the nucellar cap. The body cell has divided to give two sperm cells. The papillae, which are the remains of the exine, are very prominent at the apices of the tubes. 29 Jan., 1939.  $\times 29$ .

Text-fig. 51.—Part of a pollen tube exposed in a longitudinal section of the nucellar cap. It shows the tube-nucleus surrounded by an aggregation of starch grains, the two sperm cells towards the free end, each with a much enlarged nucleus, portions of the developing spiral band in section, and at tip, the prothallial nucleus also amid starch grains. 21 Jan., 1939.  $\times 116$ .

Text-fig. 52.—Longitudinal section of tip of the generative region of male gametophyte showing the two large immature sperm cells. The series of furrows caused by the spiral band are obvious in the wall and from these deeply-staining tufts of short cilia project. 10 Jan., 1939.  $\times 268$ .



Text-fig. 53.—Dorsal view of part of the apical region of the nucellar cap. In the centre is the pollen chamber and radiating therefrom a series of long, branching haustorial tubes of male gametophytes, permeating the tissue of the nucellus. The central region of the nucellus is typically cone-like with a crater-like opening in the middle. Drawn with a binocular microscope from living specimen. 31 Dec., 1938.  $\times 24$ .

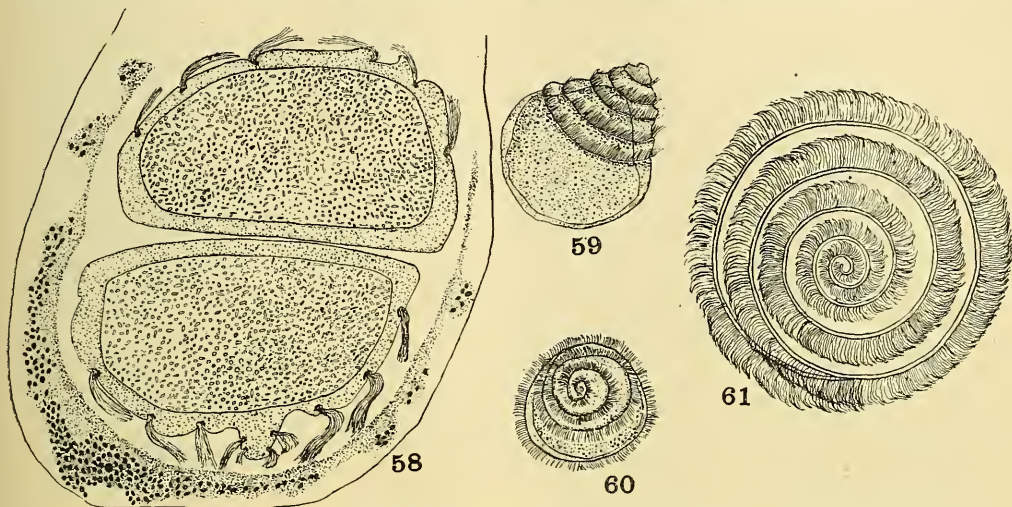
Text-fig. 54.—Ventral view of the nucellar cap described in previous figure. The lateral gametophytic haustoria can be seen through the thin nucellus whilst pendent in the centre are the generative ends of the pollen tubes. These are dilated, club-shaped structures projecting in close formation beyond the narrow passage of the broken-down pollen chamber, and protruding slightly into the archegonial chamber. Each shows a small papilla-like projection at the tip, which marks the position of the original microspore. The contents are indistinct, but the motile sperm stage has not been reached. 10 Jan., 1939.  $\times 20$ .

Text-fig. 55.—A stage slightly more advanced than that depicted in preceding figure. In one tube motile sperms were seen, but the majority of the others had shed their sperms and were devoid of contents. 10 Jan., 1939.  $\times 24$ .

Text-fig. 56.—Another view of the ventral surface of the nucellus with the generative regions of upwards of a score of pollen tubes now extended deeply into the archegonial chamber. The nucellus is greatly attenuated, its substance having been absorbed by the haustoria. A body-cell is located near the protruding tip of each tube. 21 Jan., 1939.  $\times 24$ .

Text-fig. 57.—Side view of a nucellar cap similar to those illustrated in preceding figures. It shows to better advantage the depth to which the sperm-bearing ends of the pollen tubes hang below the surface of the nucellus after penetrating the pollen chamber. Sperms are present near tips of tubes. Drawn from living material with binocular microscope. 31 Jan., 1939.  $\times 24$ .

material in the third week of January, and examined under the binocular microscope, presented the arresting spectacle of the active sperms swimming upward and downward in the liquid contained in the pendent region of the tube. The sperms moved forward, and at the same time each rotated on its axis. While under observation they retained their activity for varying periods up to ten minutes (Text-figs. 55 and 57). Drawings made after the quiescent stage had been reached are shown in Text-figures 59 and 60. The appearance of a living sperm with five coils in its ciliated band is portrayed, while a



Text-fig. 58.—Longitudinal section of generative tip of pollen tube containing two sperm cells, almost mature. The furrows caused by the spiral band are now very deep, and the long densely-arranged cilia project freely. Abundant starch grains are embedded in the surrounding cytoplasm. 21 Jan., 1939.  $\times 365$ .

Text-fig. 59.—Lateral view of a living sperm. Its pyramidal shape and spiral ciliated band are obvious. This sperm was forced out of the pollen tube and for a short time swam freely in the liquid exuded. The huge nucleus is almost spherical. Drawn with binocular microscope. 31 Jan., 1939.  $\times 75$ .

Text-fig. 60.—Surface view of a living sperm. The symmetrical coiling of the spiral band which executes five complete turns is remarkably clear. The closely set cilia vibrate with great rapidity. Drawn with binocular microscope. 31 Jan., 1939.  $\times 75$ .

Text-fig. 61.—A high-power study of a sperm in surface view. This illustrates the length of the cilia and their arrangement on the spirally-coiled band. 21 Jan., 1939.  $\times 235$ .

surface view from a stained preparation is depicted in Text-figure 61. The appearance of the young sperms in section, soon after their formation, each with its large nucleus and spiral ciliated band, is indicated in Text-figure 52, while the general contents of the pollen tube, namely, starch grains, tube-nucleus, mature sperms and stalk-cell nucleus, are featured in the text-figure immediately preceding. The structure of the mature sperms, now free from each other, is better illustrated in Text-figure 58, where the grooves due to the pressure of the ciliated band are more pronounced. As already stated, pollination occurs normally during the latter half of October and early November, while fertilization is not effected until mid-January, so that a period of a little over two months elapses between these two significant events.

#### *Fertilization.*

The thin nucellar cap fits over the archegonial chamber, forming a compartment sealed off from the outer air (Text-figs. 38 and 53). Pollen tubes removed from this chamber, and exposed to the external atmosphere with a high relative humidity and a temperature of 80° Fahrenheit, showed immediate symptoms of desiccation, thereby indicating that the moisture content of the internal atmosphere must be saturated. In support of this theory it was noted that the walls of the chamber were moist. It follows, therefore, that in more mature ovules, where the contents of the pollen tubes have been

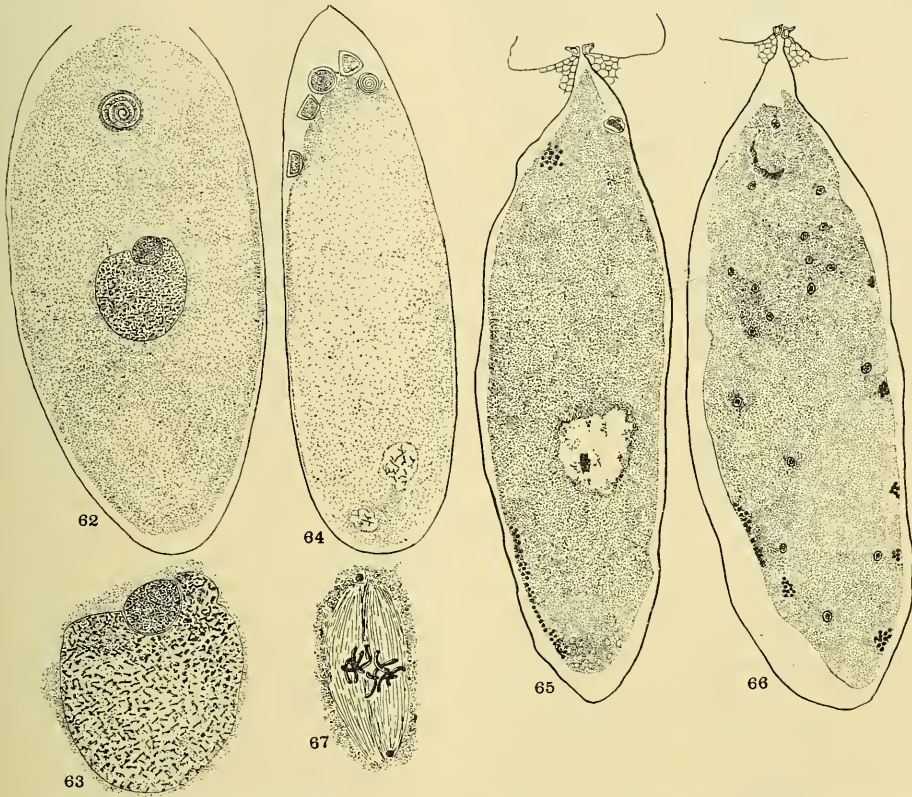
ejected into such an environment, there is little chance of desiccation of the sperms even though their passage through the neck cells into the egg be not immediately effected. The quantity of this fluid must be considerable, seeing that as many as twenty pollen tubes may discharge into one chamber, and in consequence provide sufficient liquid to submerge the exceptionally large sperms. Lawson (1926) contends that the neck-cells "actually secrete water after the manner of superficial hydathodes", and that the sperms can and do swim within the archegonial chamber. It may be pointed out, however, that the amount of free water secreted could not be very large, otherwise it would dilute the liquid ejected from the pollen tubes, and thus by osmosis increase the internal pressure, of the sperms to a harmful extent. The neck-cells are a conspicuous feature of the mature archegonium (Text-figs. 32 and 33). The writers have not found in *Macrozamia* the differential thickening of the walls of the neck-cells which Lawson figures in *Bowenia*, and if this particular structural feature is a necessary condition to his explanation of the opening mechanism for the passage of sperms, such a method is inoperative in *Macrozamia*. In Text-figure 32, it may be seen that the part of the adjoining walls actually in contact at the period just prior to fertilization is limited. Accordingly, it is easily conceivable that at the precise period when sperms are free in the archegonial chamber, very little modification in the shape of the neck-cells—owing to some change in their turgidity—in conjunction with the upward thrust of the extremely turgid contents of the egg would lead to their actual separation. Even though this passage be less than  $215\mu$  (the average diameter of the sperm), an entry may be effected either by the slight force exerted by the sperm or by modification in its shape, since such fluctuation in form has already been noted while the sperm was still swimming in the pollen tube. No matter what the correct explanation may be, the fact remains that the mechanism is most efficient, since in many cases several sperms gain entry to a single egg.

Considering that a score of pollen tubes and five archegonia are not uncommon in one chamber, that is, a ratio of eight sperms to each egg, it is not surprising that numerous sperms may enter any one egg. In Text-figure 64 five sperms, in addition to the functional one, have passed through the neck-cells. These superfluous sperms degenerate but slowly, and are easily recognizable even in the early stages of development of the embryo, albeit the cilia are no longer apparent. The neck-cells appear to be unaltered by the entry of these sperms, since examination at the post-fertilization stage showed no malformation. The functional sperm escapes from its sheath which is left in cytoplasm near the periphery of the egg, the ciliated band being an easily recognizable feature, however, until wall-formation in the proembryo. The male nucleus gradually moves towards the egg nucleus with which it finally makes contact, their membranes, however, remaining intact. The disparity in size of the two nuclei is striking (Text-fig. 62). Each contains a large amount of a deeply-staining substance which, as Chamberlain (1935) points out, has never been satisfactorily interpreted, and this constitutes the "metaplasm" of Strasburger. Even at the stage when the male nucleus has become practically engulfed in the egg nucleus, nothing which could be diagnosed as chromatin was observed (Text-fig. 63).

#### *The Embryo.*

After fertilization, during which contact of the sperm and egg and the subsequent union of their nuclei have resulted in the formation of the zygote, development proceeds rapidly. The earliest clearly recognized stage in embryogeny was the metaphase of the nuclear division of the zygote (Text-fig. 65). The spindle takes the position formerly occupied by the nucleus of the egg, that is, just within the lower half of the egg-sac, and is surrounded by a large clear region, referred to by Chamberlain (1935) as the "fibrillar area", and containing a few widely separated cytoplasmic strands somewhat akin to spindle fibres. The number of chromosomes identified during a study of consecutive sections through two different spindles of the dividing zygote nucleus conforms with the number eighteen ascertained for *Macrozamia Miquelii*, *Macrozamia Moorei* and *Macrozamia tridentata* by Sax and Beale (1934). The double structure of the fusion spindle described by Lawson (1926) for *Bowenia* was not positively identified in

the present case, but the rather broad blunt-ended spindle of this first division does suggest a dual nature (Text-fig. 67). However, later stages fail to show the double spindle which Lawson states persists "up to the last mitosis forming free nuclei in the proembryo". Sections through the proembryo of *Macrozamia spiralis* show that



Text-fig. 62.—A longitudinal section through an archegonium during the period of fertilization. The actual fusion of the sperm nucleus with that of the egg is depicted. The membrane of each nucleus is still intact, the ciliated spiral band of the male nucleus being left behind in the upper cytoplasm at the time when the nucleus escaped from its sheath. The cytoplasm is contracted from the apical region of the egg, leaving a hyaline region filled with liquid. 21 Jan., 1939.  $\times 28$ .

Text-fig. 63.—High-power study of the union of the male and female nuclei; the former is almost entirely enveloped in the metaplastm of the egg nucleus. The contents of the sperm nucleus are finely granular, while those of the egg are exceedingly coarse in comparison. 21 Jan., 1939.  $\times 58$ .

Text-fig. 64.—Longitudinal section of an egg. Five sperms have effected an entrance and impinge on the egg cytoplasm, between the margin of which and the apex is the characteristic hyaline region. One of the sperms with its ciliated band is partly buried in the cytoplasm but the others, which stain more deeply, appear to be degenerating. At the lower end of the egg two protein vacuoles occur but the egg-nucleus is not included in this section. 21 Jan., 1939.  $\times 28$ .

Text-fig. 65.—Median longitudinal section through an archegonium soon after fertilization, showing the metaphase spindle of the division of the zygote nucleus. It is surrounded by a clear fibrillar zone; also in evidence are an accumulation of starch grains towards base of egg, the collapsed neck-cells, an intact egg-sac membrane and a non-functional sperm impinging on the upper cytoplasm. 29 Jan., 1939.  $\times 33$ .

Text-fig. 66.—Early nuclear division in the proembryo showing a number of intranuclear spindles, scattered generally throughout the granular cytoplasm; the remains of the ciliated band of the functional sperm are embedded in the upper cytoplasm. 29 Jan., 1939.  $\times 33$ .

Text-fig. 67.—Highly magnified view of spindle of previous figure showing some of the chromosomes, also the blunt ends and suggestion of dual nature of the spindle. 29 Jan., 1939.  $\times 355$ .

simultaneous divisions of the nuclei do occur, and that a number of free nuclei are distributed uniformly throughout the cytoplasm (Text-fig. 66). Such divisions are intra-nuclear, the membrane being a conspicuous feature until very late stages in free nuclear division. In the upper cytoplasm, the discarded, coiled, spiral sheath of the functional sperm still persists, and even the fine cilia are distinctly visible. By this time the archegonial neck-cells have collapsed, but the egg membrane has thickened very considerably and forms a clearly delineated boundary to the developing proembryo. So far, uniform distribution of the nuclei has been maintained, but on reaching the 128-nucleate stage there is a definite tendency for the majority of the nuclei to segregate at the lower end of the proembryo. The abundance of starch grains in this region may indicate a general "settling", as pointed out by Coulter and Chamberlain (1903) in the case of *Zamia*, but no definite cytoplasmic strands were diagnosed (Text-fig. 68).

It is probable, too, that in the lower region subsequent divisions of the nuclei proceed more rapidly: certainly wall formation is initiated here earlier than in the upper part. Wall formation commences with the development of a peripheral layer of cells and a centripetal growth of the tissue thus initiated (Text-fig. 69). Again, development proceeds more rapidly at the lower end. No sign of the formation of evanescent walls prior to true wall formation, such as has been observed for *Dioon* and *Stangeria* (Chamberlain, 1935), was observed in the case of *Macrozamia spiralis*. After the free nuclear stage the proembryo becomes cellular throughout and in this respect agrees with "some species of *Cycas*, *Encephalartos*, and *Macrozamia*" (Chamberlain, 1935), but differs from *Macrozamia Reidlei* (Baird, 1939) in which there is "a small region in the centre, which remains non-cellular with free nuclei" and which "breaks down about the time the embryo is beginning to differentiate". Furthermore, in the embryogeny of *Macrozamia spiralis* the completely cellular condition once attained is a persistent feature, and at no subsequent stage is there in the central region that breaking-down of tissue which is so characteristic of those other cycads in which the early proembryo is recorded as being cellular throughout. Subsequent development leads to increase in size of the proembryo, but growth continues to be more vigorous at the lower end, where a mass of small densely cytoplasmic cells become segregated (Text-fig. 70). The cells of this region are in marked contrast to the larger vacuolate cells of the rest of the proembryo.

The next phase of importance is marked by the elongation of the cells comprising the four or five tiers immediately behind the actively meristematic zone (Text-fig. 71). The young suspensor, thus initiated, grows rapidly, particularly in length, so that the embryonic apex is forced downwards, eventually ruptures the tough egg-membrane (Text-fig. 72 and 73), and makes direct contact with the endosperm. Thereupon, enzyme action (Pl. xvii, fig. 23) results in the provision of an abundant food supply for the nourishment of a massive embryo (Text-fig. 73).

Elongation of the suspensor, however, greatly exceeds the actual distance penetrated into the endosperm. Consequently, the suspensor becomes coiled and twisted. It finds

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Text-fig. 71.—In this longitudinal section, which is not quite median, differentiation of the suspensor cells has been initiated, the cells immediately behind the meristematic zone showing vacuolation and elongation. The general increase in length of the proembryo has caused the sac membrane to rupture in the region adjacent to the meristem. 2 March, 1939.  $\times 22$ .

Text-fig. 72.—A still older stage of the proembryo; the suspensor is clearly established, and has thrust the embryo through the membrane and into the endosperm tissue. 5 March, 1939.  $\times 33$ .

Text-fig. 73.—A representation of a still older stage in the development of the proembryo. The suspensor cells have increased greatly in length and the embryo proper has been forced deep into the tissue of the endosperm. The surrounding clear zone is due to enzyme secretion by the embryo with consequent digestion of the contiguous cells. 5 March, 1939.  $\times 33$ .

Text-fig. 74.—High-power representation of a longitudinal section of the lower part of a young suspensor, and terminal meristem. The cells of the latter are densely cytoplasmic, and their meristematic activity is attested by the numerous nuclei undergoing division. 5 March, 1939.  $\times 355$ .



Text-fig. 68.—Stage of development of proembryo somewhat later than that portrayed in Text-figure 66. Further nuclear divisions have occurred, and about ninety-six nuclei are now visible in the cytoplasm of the proembryo. These nuclei tend to congregate at the base of the sac, while the amount of starch has increased, especially at the lower end. The membrane of the sac is still intact and a non-functional sperm lies within the collapsed neck-cells at the upper end. 31 Jan., 1939.  $\times 33$ .

Text-fig. 69.—This longitudinal section through the developing proembryo shows the initiation of true cell-walls. Around the periphery the cells are smaller and more crowded towards the lower region. Numerous free nuclei embedded in cytoplasm still occupy the central region. 31 Jan., 1939.  $\times 33$ .

Text-fig. 70.—Median longitudinal section of young proembryo which has now become completely cellular. The differentiation of a meristematic zone at the base of the sac is evident, while larger vacuolate cells occupy the central and upper parts. 5 March, 1939.  $\times 33$ .

accommodation for an increasing bulk by breaking down the cells and filling the space formerly occupied by the relatively extensive and soft tissue of the upper region of the proembryo. All the eggs of a female gametophyte may be fertilized, and any or all of the resulting zygotes may develop vigorously (Text-fig. 80). Consequently a young seed usually contains an endosperm into which several embryos with long suspensors have burrowed. So long as these suspensors are fairly straight the embryos concerned may easily be dissected one from another (Pl. xvii, figs. 21 and 22), but subsequent elongation and coiling in a restricted space results in the suspensors becoming inextricably intermingled within the common cavity formed from the space formerly occupied by the egg and the partial disintegration of the endosperm (Pl. xvii, figs. 19 and 20).

At this point it is interesting to observe that the several tough egg-membranes still persist in the seed, and may be recognized as balloon-like structures, each enclosing the upper portion of a young sporophyte (Text-fig. 80). Of course, such membranes are later crushed by the enlarging embryos. Lawson (1926), in describing the embryology of *Bowenia*, has stated that the suspensors may fuse together at different points. The present investigation does not support the idea of anything in the nature of organic union but rather that, as Chamberlain (1935) has explained, they are intimately associated and form a composite body until, during later development, one embryo after another ceases growth, and is left behind until only one survives. The suspensor of this embryo, however, persists, and may reach at maturity a length, when extended, of 9.5 centimetres (Text-fig. 83), by which time all the primary organs of the embryo have been differentiated. But in *Macrozamia spiralis* a more novel and interesting method of increasing the number of embryos in any one seed has been observed. The condition has been attained through budding of the embryo, so that another kind of polyembryony arises. It originates in a manner totally different from that described above for *Macrozamia spiralis* in particular, and for cycads in general. Dissection, and examination of sections, of young seeds showed that several embryos may arise within a single egg-membrane. For example, in a single seed collected during February, when the embryos are still sufficiently young to be easily separated, five out of the six embryos from different archegonia possessed either two or three meristems. In three of the cases the lateral meristems were far removed from the basal meristematic zone (Text-figs. 77, 78 and 79), but in one case two growing points, located side by side, occupied the lower extremity of the embryo (Text-fig. 76). Some of these embryos had already developed short suspensors. Baird (1939) has drawn attention to the occurrence of such embryos in *Macrozamia Reidlei*.

Reference to Text-figures 75 to 79 shows where and how such supernumerary individuals arise. It is clear that the superficial cells in any region of the proembryo—except perhaps that portion adjoining the neck-cells—may become meristematic, and that the actively dividing zones thus formed rapidly develop the essential features of the normal embryo. Such embryos grow out in all directions and eventually impinge on, and penetrate, the egg-membrane. In some cases the direction of further growth is deflected by resistance of the tough membrane, and then the tips usually turn downwards in the direction of the rupture effected by the first-formed and normal embryo. Some embryos, however, are deflected in an upward direction and provide the striking spectacle of young sporophytes growing in the direction of the micropyle. However, none of the inverted embryos observed developed sufficiently to permit of their passing beyond the limits of the archegonium.

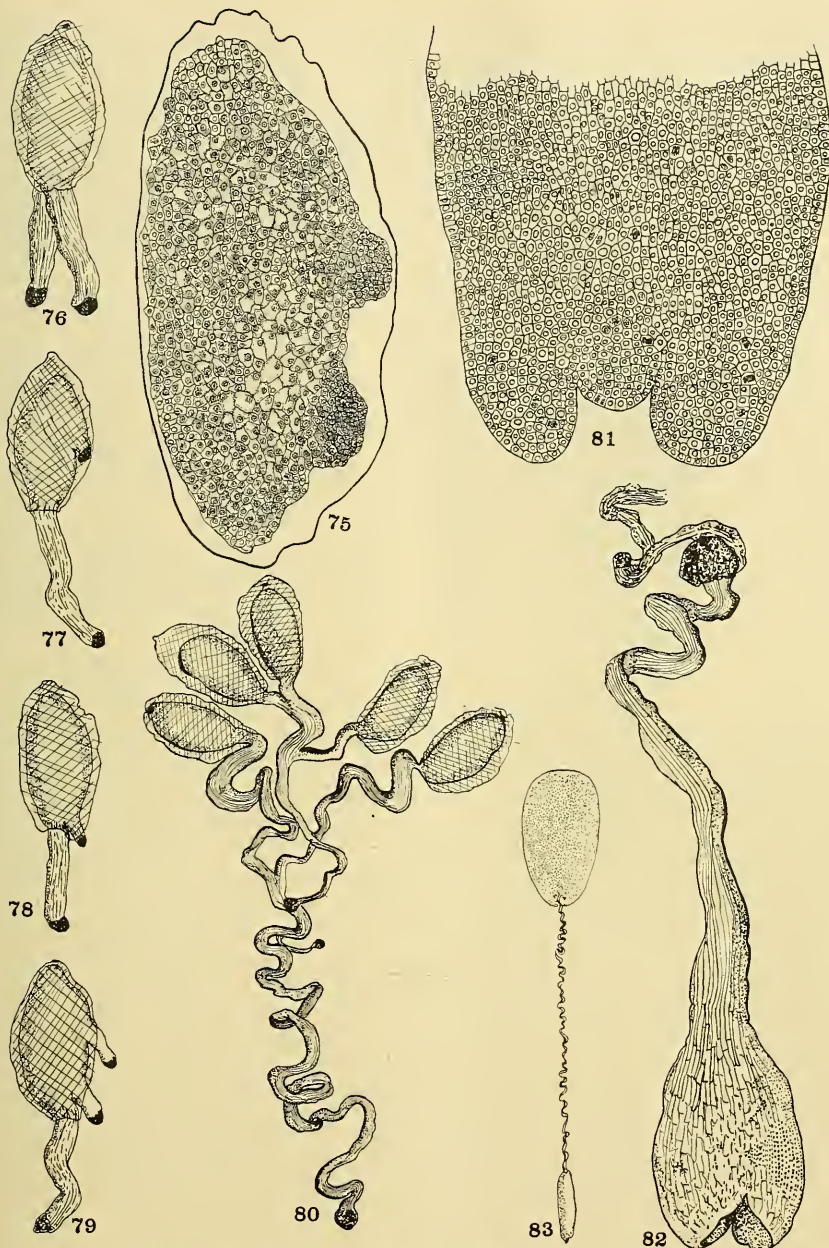
Branching of the suspensor has been recorded in *Encephalartos* by Saxton (1910). In the present investigation two well-developed embryos of approximately the same

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Text-fig. 81.—Median longitudinal section of early cotyledon stage of the embryo; the stem tip is terminal and the two cotyledons lateral. 11 March, 1938.  $\times 96$ .

Text-fig. 82.—A slightly older embryo than that depicted in previous figure. Two broad fleshy cotyledons are evident. The region behind the cotyledons gives rise to the coleorhiza. 12 May, 1939.  $\times 8.4$ .

Text-fig. 83.—A megascopic view of endosperm of a seed dissected to show the extent of the coiled suspensor and the large embryo proper attached. 7 June, 1939.  $\times 0.4$ .



Text-fig. 75.—This diagram shows a longitudinal section through a proembryo, possessing lateral meristems as well as a normal basal meristem; the latter is not included in this drawing but was observed in succeeding sections of the series. In no case have suspensor cells been initiated. 5 March, 1939.  $\times 40$ .

Text-figs. 76 to 79.—Diagrams 76 to 79 illustrate polyembryony due to sporophytic budding, and embody a series of proembryos dissected from a single seed. In figure 76 a lateral embryo is seen alongside the terminal one; in figure 77 the relatively undeveloped lateral embryo is still within the egg membrane; in figure 78 a lateral embryo has penetrated the egg membrane, while the succeeding figure depicts a proembryo with two lateral individuals originating at different levels. In all cases a suspensor has been developed, but these vary considerably in length. 18 Feb., 1940.  $\times 7.2$ .

Text-fig. 80.—This diagram illustrates five embryos derived from five different archegonia. The coiled suspensors are closely intertwined, and all but one of the embryos have aborted. The persistent egg membranes are conspicuous. 13 April, 1939.  $\times 3.6$ .

dimensions were occasionally found in the position usually occupied by the normal embryo, an occurrence which naturally suggested an origin identical with that claimed in *Encephalartos*. However, the study of suitable material at earlier stages showed that a secondary meristematic zone may be found close to, and very soon after, the inception of the first-formed apical meristematic tip. In this case, seeing that no suspensor has yet been developed, branching of the suspensor as the cause of polyembryony is clearly excluded, and in *Macrozamia* no evidence of such a happening has so far come to light. The known facts indicate that embryos arise independently from the undifferentiated proembryo, although the possibility of increase by division of the apical meristem itself is not precluded.

As already indicated the budded embryos abort relatively early and, in seeds containing an advanced embryo, no trace of their presence was discovered.

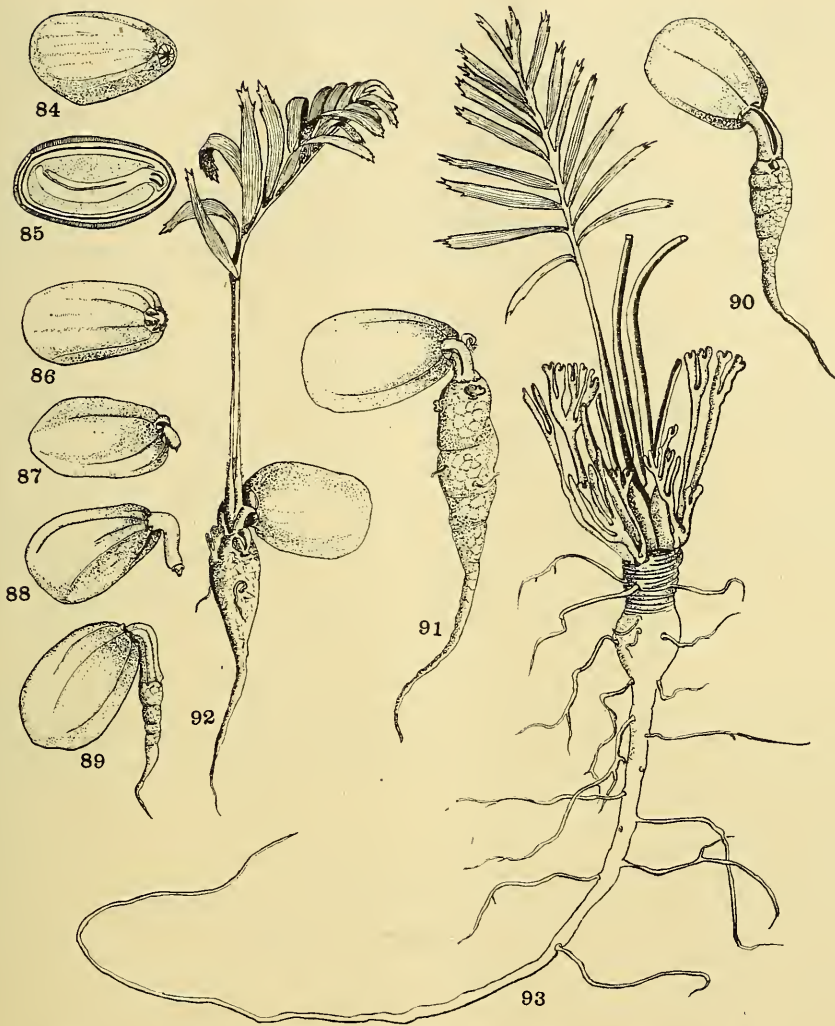
Eventually, all but one of the embryos which may have commenced development in any one seed become crushed and absorbed by the functional one which slowly but steadily invades the endosperm and increases in size. Microtome sections of the endosperm of seeds collected in March showed the epicotyl and the primordia of the two cotyledons (Text-fig. 81). The latter grow rapidly until in May they appear as relatively large, fleshy organs as represented in Text-figure 82.

By late June the epicotyl, cotyledons, hypocotyl and root of the embryo are recognizable. Thereafter a steady increase in size of the various parts proceeds until material examined in December reveals an embryo about 2·3 centimetres in length attached to a suspensor which, even when slightly coiled, extends to a length of 9 centimetres (Text-fig. 83).

#### *Germination and the Seedling.*

Subsequent to fertilization the axis of the cone slowly elongates. Thereby the megasporophylls are separated, the seeds become exposed, and are conspicuous on account of their vivid colour which in different cones varies from yellow to pink or deep red. Meanwhile a gradual process of desiccation is operative, and the formation of an abscission layer near the base of each sporophyll eventually leads to its falling away from the cone axis, and the deposition of a colourful mass of seeds around the bare and protruding peduncle. Such seeds gradually become freed from the sporophylls, and are found scattered irregularly around the base of the plant.

Under normal conditions the seed does not undergo any resting condition and by April or May, some fifteen or sixteen months after fertilization, germination may occur. At such time the soft coloured outer coat of the testa has either dried to a thin irregular layer or has completely decayed, leaving the stony layer exposed. In the region of the micropyle radiating cracks appear (Text-figs. 84 and 85). These fissures are due in particular to pressure exerted by the expanding coleorhiza, but also to the increasing bulk of the embryo in general. Gradually that portion of the integumentary zone which encircles the micropyle is split up into widely separated wedge-shaped masses (Text-fig. 86) from the centre of which the greenish coleorhiza protrudes (Text-fig. 87), turns downwards, and penetrates the soil. Its elongation anchors the seedling to the soil by which time the tip of the primary root becomes apparent at the free end of the coleorhiza, its emergence being effected partly by force. The ruptured coleorhizal tip is split into wedge-shaped segments similar to those already encountered at the micropylar end of the testa (Text-fig. 88). The root grows quickly at the expense of the stored material within the endosperm, becoming fleshy and stout in outline, and wrinkled on the surface (Text-figs. 89 and 90). The appearance of secondary roots follows, but certain of these located on the upper contractile region of the primary root are highly modified and appear as dichotomously branched, coralloid masses (Text-figs. 91-93). These are apogeotropic in nature and usually occur in two groups on opposite sides of the tap-root, the surface of which has, meanwhile, become uneven and roughly patterned, owing in part to the formation of a protective periderm. The young sporophyte frees itself from the testa until all but the tips of the cotyledons



Text-figs. 84-93.—Series of drawings illustrating the germination of the seed and establishment of the seedling. Figure 85 represents a seed in longitudinal section and shows the hard outer coat of the testa, the membranous remains of the nucellus, a bulky endosperm enclosing an embryo in which the separate cotyledons and young root within the coleorhiza are depicted. For further details see text. The serrated nature of the pinnae is evident (figures 92 and 93); this character is absent from older plants.  $\times 8/15$ .

are withdrawn (Text-fig. 90). The latter, acting as haustorial organs, draw on the food supply stored in the endosperm, until this is exhausted, when the empty shell of the testa and the withered cotyledons become detached. During this period also the curved outline of the first leaf, covered by protective hairs, appears between the exposed parts of the two cotyledons (Text-fig. 91).

Subsequently, the root system continues to develop, the tap root extending to a length of some fifteen inches, while at its thickest part a diameter of one and a half inches is attained (Text-fig. 93). At the same time, secondary and tertiary roots have multiplied, the coralloid mycorrhizal system being specially massive, much branched, and protruding above ground.

Such structures are an outstanding feature of a seedling during the first few years of its growth, but, although they persist, they are relatively inconspicuous in the

mature plant. In transverse section an "algal zone" is easily discernible to the naked eye, although the prominence of this area varies with the edaphic conditions, being specially well-formed in plants growing in rich, loamy soils, or under cultivation in gardens or glass houses. The appearance of the second leaf follows about a month after the first has unfolded; thereafter leaf production is exceedingly slow and only five or six leaves may arise in the first three years of growth.

A conspicuous feature of the pinnae of young plants is the serrations found near their tips (Text-figs. 92 and 93). These, however, are evanescent and gradually disappear with increasing maturity of the plants which, on reaching the age of ten to twelve years, are quite devoid of such vestiges. The presence of these features in young plants is regarded as evidence that the serrated margin was a permanent structure in the ancestral form of *Macrozamia spiralis*.

A survey of the facts disclosed by this investigation shows that certain peculiar features, namely, the archegonial neck-cells occasionally being in excess of two, and polyembryony due to sporophytic budding of the embryo, are common to *Macrozamia* and *Encephalartos* (Saxton, 1910). In addition, the average number of sporangia per sporophyll in *Macrozamia spiralis* is 342, and in *Macrozamia Miquelii* 503 (Chamberlain, 1935, p. 115). The mean of these numbers, 422, lies between those given for *Encephalartos caffer*, 567, and *Dioon edule*, 295, as supplied in Chamberlain's list. Again, in *Encephalartos* and *Macrozamia* (Coulter and Chamberlain, 1917) numerous vascular bundles are found in the pith, while all cones are lateral, and stem growth is monopodial.

The above features taken in conjunction point to the conclusion that *Macrozamia* has a closer affinity with *Encephalartos* than with any other cycadean genus.

#### SUMMARY.

*Macrozamia spiralis* is distributed along some one thousand miles of the south-eastern coastal region of Australia. The body-form is modified by the prevailing edaphic conditions and the operation of contractile roots. Cones always arise laterally. Cones weighing as little as 0.6 gram were examined and by using certain specified precautions in the *modus operandi* these minute strobili may be detected and removed without destroying the plant.

The comparative rates of development in the component parts of the staminate and ovulate cones are presented in tabulated form.

The division of a deep-seated megaspore mother-cell results in the formation of three cells; the chalazal one persists as the functional megaspore.

The development of the female gametophyte is normal. Irregularly-shaped or malformed archegonial chambers occasionally occur.

The development of the microsporangium exhibits no unusual features.

The slit of dehiscence is defined by a band of thin-walled tissue, which is four cells in breadth. Pollination is anemophilous.

The growth of the male gametophyte is normal for cycads. Evidence is presented to show that the atmosphere of the archegonial chamber is saturated with moisture. Prior to fertilization the sperms swim actively in the pendent generative region of the male gametophyte, and are subsequently ejected into the archegonial chamber, whence they pass between the greatly dilated neck-cells into the egg. In some ovules the neck of the archegonium is comprised of four cells. An interval of two months intervenes between pollination and fertilization.

The method of entry by the sperms into the egg is discussed. Numerous male gametes may enter one egg. The behaviour of the male and female nuclei during fertilization is described. The division of the nucleus of the zygote was observed. Free simultaneous nuclear division follows; later, wall-formation is initiated.

At maturity the proembryo is cellular throughout, and remains so. Polyembryony arises from two causes: first, by the fertilization of more than one egg in a single female prothallus, and second, by sporophytic budding from the proembryo, almost any cells of which may become actively meristematic and give rise to an embryo. Only one embryo in a seed reaches maturity.

The seed germinates without any resting period, and produces a seedling having a contractile tap root, bearing abundant apogeotropic roots with prominent algal zone.

The results of this investigation substantiate the view that *Macrozamia* is more closely related to *Encephalartos* than to any other cycadean genus.

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#### EXPLANATION OF PLATES XIV-XVII.

##### Plate xiv.

- 1.—General view of a dense society of *Macrozamia spiralis* in open eucalyptus forest. 10 Jan., 1939. Narooma, N.S.W.
- 2.—Seed plant of *Macrozamia spiralis*. A few leaves have been excised in order to expose two cones, almost mature, and averaging ten and three quarter pounds in weight. The leaf bases and organic apex are buried in the soil. 10 Jan., 1939. Narooma, N.S.W.
- 3.—Microsporangiate plant of *Macrozamia spiralis* bearing ten mature cones, seven of which are visible. 10 Jan., 1939. Narooma, N.S.W.
- 4.—Single plant growing in a quartzite soil with trunk protruding some thirty inches above ground. 10 Jan., 1939. Dalmeny, N.S.W.
- 5.—Part of plant showing stem rising almost three feet above soil level, and enclosed in an armature of tough leaf bases. 10 Jan., 1939. Dalmeny, N.S.W.
- 6.—Apex of trunk partly cut away to show young leaves and position of organic apex. 10 Jan., 1939. Dalmeny, N.S.W.

##### Plate xv.

- 7.—Immature megasporangiate cone showing sporophylls, each with characteristic prolonged upturned process, and exposed ovules, which are marginal in position. 29 Dec., 1939.
- 8.—Mature megasporangiate (a) and microsporangiate (b) cones compared. The pollen has been shed from the latter. 27 Jan., 1939.

9.—Series of megasporophylls (*E-I*) and microsporophylls (*e-h*) showing relative sizes and shapes at intervals throughout development. *Dates of collection*: *E*, 14/4/38; *F*, 6/7/38; *G*, 4/11/38; *H*, 8/12/38; *I*, 22/2/39; *e*, 12/6/38; *f*, 4/10/38; *g*, 4/11/38; *h*, 20/12/38.  $\times 0.42$ .

10.—Two microsporophylls from previous figure. *a*, just prior to dehiscence, and *b*, after dehiscence. In (*a*) the soral arrangement and line of dehiscence of the sporangia are clearly shown, while in (*b*) the sporangia gape widely and have shed their spores.

#### Plate xvi.

11.—Central apical region removed from a seed plant. The organic apex has produced, and is hidden amid numerous yellowish-white leaves, while laterally are situated three bud-like structures, each consisting of brown, furry, protective leaves encasing a strobilus. 3 Feb., 1940.

12.—Bud with some of the outer proximal leaves removed in order to expose the minute enclosed ovulate cone, which weighed 1.3 grams. The megaspore mother-cell was present in this material. 20 Jan., 1940.

13.—Microsporangiate cone, weight 11.0 grams. 3 Feb., 1940.

14.—Megasporangiate cone enclosed by the younger protective leaves. Feb. 8, 1940.

15.—Series of young megasporophylls (*A-D*) and young microsporophylls (*a-d*) showing comparative rates of development. *Dates of collection*: *A*, 20/1/38; *B*, 9/2/38; *C*, 11/3/38; *D*, 7/5/38; *a*, 20/1/38; *b*, 3/2/38; *c*, 11/3/38; *d*, 2/4/38.

16.—Photomicrograph of male nucleus almost enveloped by the nucleus of the egg. The finely granular nature of the former as compared with the coarsely granular appearance of the latter is evident. 10 March, 1939.  $\times 117$ .

#### Plate xvii.

17.—Longitudinal section through female gametophyte showing deep archegonial chamber, and evidence of several proembryos with long coiled suspensors; the meristematic tips have burst through the egg membrane and are invading the endosperm. 10 March, 1939.  $\times 7\frac{1}{2}$ .

18.—Section somewhat similar to that immediately preceding, but illustrating more clearly the lower part of a suspensor and an apical meristem. The clear region in the endosperm adjoining the egg membrane shows a notable absence of starch: this food reserve has been used to nourish the developing proembryos. 10 March, 1939.  $\times 13\frac{1}{2}$ .

19.—High-power study of portion of above figure to show the elongated cells of the suspensor, and the apical meristem. 10 March, 1939.  $\times 28$ .

20.—Apical meristem under still higher power of magnification. The invasion of the endosperm by the enzyme activity of the densely cytoplasmic cells of the meristem is clearly revealed. 10 March, 1939.  $\times 133$ .

21.—Two active meristems growing side by side, and occupying the lowermost region of the proembryo. 10 March, 1939.  $\times 200$ .

22.—Longitudinal section of part of female gametophyte showing parallel development of five embryos which have originated in different archegonia. The gradual disappearance of starch from the endosperm cells around these embryos is very apparent. 10 March, 1939.  $\times 30$ .